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## EXPERIMENTAL OBSERVATIONS OF THE HYDRODYNAMIC BEHAVIOR OF SOLVENT SYSTEMS IN HIGH-SPEED COUNTER-CURRENT CHROMATOGRAPHY

### II. PHASE DISTRIBUTION DIAGRAMS FOR HELICAL AND SPIRAL COLUMNS

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#### SUMMARY

Extensive studies have been performed on hydrodynamic distribution of two immiscible solvent phases in helical and spiral columns by means of coil planet centrifugation. Under the Scheme IV [see Fig. 1 in Y. Ito, *J. Chromatogr.*, 301 (1984) 377] planetary motion, the two phases display unilateral distribution in the coil, *i.e.*, one phase travels toward the head and the other phase toward the tail. This hydrodynamic trend is sensitively affected by various factors such as physical properties of the solvent system, helical diameter of the column, revolutional radius, etc. Phase distribution diagrams obtained from a set of conventional two-phase solvent systems under various experimental conditions provide extremely useful information for both basic hydrodynamic studies and practical application in high-speed counter-current chromatography.

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#### INTRODUCTION

High-speed counter-current chromatography (CCC) utilizes a unique hydrodynamic distribution of two immiscible solvent phases in a rotating coiled column<sup>1-5</sup>. In Part I (see ref. 6), a series of preliminary studies of the hydrodynamic distribution of the two-phase solvent systems in a helical column has been described which employed the combined horizontal flow-through coil planet centrifuge which provides two different types of synchronous planetary motions, Schemes I and IV as illustrated in Fig. 2 of Part I. Phase distribution diagrams obtained from a set of solvent systems revealed that the Scheme I planetary motion produced low retention of the stationary phase whereas the Scheme IV planetary motion yielded favorable stationary phase retention under the identical experimental conditions. With the Scheme IV planetary motion, all two-phase solvent systems display unilateral hydrodynamic distribution, though the actual stationary phase retention profile in a helical column varies ac-

ording to the hydrophobicity and other physical properties of the organic phase.

In Part II, the basic hydrodynamic studies have been extended to include the effects of such centrifugal parameters as the radius of revolution and  $\beta$  value on the distribution of various two-phase solvent systems in both helical and spiral columns subjected to the Scheme IV planetary motion. A set of phase distribution diagrams for fifteen different types of conventional two-phase solvent systems illustrated in this article will provide a useful guide for the choice and application of solvent systems in performing high-speed CCC.

## EXPERIMENTAL

### *Apparatus*

Three different types of the horizontal flow-through coil planet centrifuges were employed in the present study. All share the common feature of producing Scheme IV planetary motion (see Fig. 2 in Part I<sup>6</sup>), while each provides a specific range for the revolutionary radius for the particular set of holders having different diameters. As described in Part I, the pattern of the centrifugal force field produced by the Scheme IV planetary motion is determined by a single parameter  $\beta = r/R$ , where  $r$  is the rotational radius (radius of the column holder) and  $R$  the revolutionary radius.

The combined horizontal flow-through coil planet centrifuge described in Fig. 3 of Part I was again used in the present experiments to obtain the retention data on the gear-driven column holder set at a 20-cm revolutionary radius. The use of holders with diameters of 5, 10 and 15 cm provided  $\beta$  values of 0.125, 0.25 and 0.375, respectively.

In two other types of the coil planet centrifuges, the pulley-driven holder was replaced by a simple counterweight. In the second apparatus, which has been successfully used for high-speed CCC<sup>3</sup>, the revolutionary radius is permanently set as 10 cm and the absence of the countershaft permits use of the same set of column holders to attain greater  $\beta$  values of 0.25, 0.5, and 0.75. In the third apparatus, the maximum available  $\beta$  value is further increased by eliminating the central stationary pipe which enables the large diameter holder to extend over the central axis of the centrifuge. The revolutionary radius of this apparatus is adjustable to four positions at 2.5, 5, 7.5 and 10 cm.

All these centrifuges are equipped with a rotary-seal-free flow-through system which allows continuous elution through the rotating column without risk of leakage of the solvent.

### *Columns*

Two types of column configuration were employed. The helical column (same as used in Part I) was prepared by tightly winding a piece of PTFE tubing, 5 m  $\times$  1.6 mm I.D. 2.4 mm O.D., around each holder forming uniform helical turns with the total capacity of about 10 ml. For the spiral column, two kinds of PTFE tubing were chosen, one measuring 1.6 mm I.D. and 2.4 mm O.D. and the other, 2.6 mm I.D. and 3.6 mm O.D. Each spiral column was prepared by winding the tubing tightly between a pair of closely spaced flanges mounted around the holder to make a single-layer flat spiral configuration. In both helical and spiral columns, the pitch of each turn is equivalent to the O.D. of the tubing.

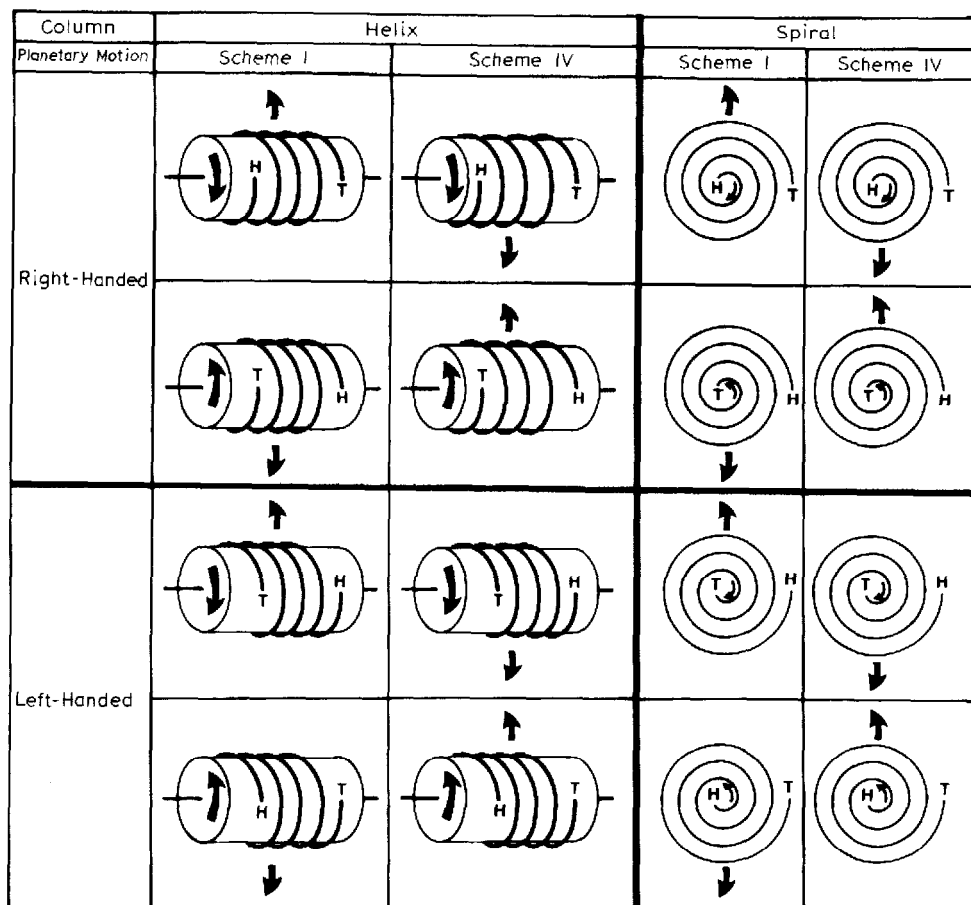


Fig. 1. Head-tail orientation of helical and spiral columns in synchronous planetary motion. H = Head, T = tail. Under the centrifugal force fields produced by the two typical synchronous planetary motions, the head-tail orientation of both columns is determined by the handedness of the coil and column rotation but is irrelevant to column revolution. Note that the handedness of a flat spiral is altered if viewed from the other side.

A coiled column has a handedness which, together with the direction of column rotation, determines Archimedean screw motion of the column contents. Under a given mode of planetary motion, any object present in the column tends to move toward one end of the column. In order to define this asymmetry of the coiled column, this end is called the head and the other end, the tail of the column. This head-tail relationship is determined by the combination of the handedness and the rotation of the column but is irrelevant to the direction of revolution as illustrated in Fig. 1. In both helical and spiral columns, the head-tail orientation determined by the planetary motion plays a major role in the hydrodynamic distribution of the two solvent phases in the column. In the spiral column, a gradient of the centrifugal force field created from the internal terminal to the external terminal of the column produces an additional effect on the phase distribution especially in the column made of larger diameter tubing which bears a greater spiral pitch.

### Solvent system

Using glass-distilled organic solvents, including hexane, ethyl acetate, chloroform, *n*-butanol, *sec.*-butanol and methanol (Burdick & Jackson Labs., Muskegon, MI, U.S.A.), glacial acetic acid of a reagent grade (Fisher Scientific, Fair Lawn, NJ, U.S.A.) and sodium chloride (J. T. Baker, Phillipsburg, NJ, U.S.A.), the following fifteen kinds of two-phase solvent systems were prepared: 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-0.1 *M* NaCl (1:1), *n*-butanol-1 *M* NaCl (1:1), *n*-butanol-acetic acid-water (4:1:5), *n*-butanol-acetic acid-0.1 *M* NaCl (4:1:5), *n*-butanol-acetic acid-1 *M* NaCl (4:1:5), *sec.*-butanol-water, *sec.*-butanol-0.1 *M* NaCl (1:1), and *sec.*-butanol-1 *M* NaCl (1:1). Among these, nine volatile solvent systems are the same as those used in the previous experiments described in Part I<sup>6</sup>.

Each solvent system was thoroughly equilibrated in a separatory funnel at room temperature and separated before use.

### Phase distribution diagram

Phase distribution diagrams for the set of two-phase solvent systems were determined for helical and spiral columns subjected to Scheme IV planetary motion under various centrifugal conditions. The experimental procedure for measuring the retention of the stationary phase and the method for drawing the phase distribution diagrams are described in the experimental section of Part I<sup>6</sup>.

For the helical column, phase retention was measured for two different elution modes, *i.e.*, the head to tail elution and the tail to head elution which were expressed on the phase distribution diagrams as solid and broken curves, respectively (Table IA).

For the spiral column, which bears the internal and external terminals, experiments were performed with four different elution modes for each mobile phase, *i.e.*, the external head to the internal tail, the internal head to the external tail, the external tail to the internal head, and the internal tail to the external head. In order

TABLE I

SYMBOLIC DESIGN OF THE RETENTION CURVES IN THE PHASE DISTRIBUTION DIAGRAM FOR HELICAL AND SPIRAL COLUMNS

H = Head; T = tail; I = internal; E = external.

|                    | Elution mode       |                    | Retention curve   |
|--------------------|--------------------|--------------------|-------------------|
| (A) Helical column | H → T              |                    | Solid line        |
|                    | H ← T              |                    | Broken line       |
|                    | Elution mode       |                    | Retention curve   |
|                    | Upper phase mobile | Lower phase mobile |                   |
| (B) Spiral column  | EH → IT            | IH → ET            | Thick solid line  |
|                    | IH → ET            | EH → IT            | Thin solid line   |
|                    | IH ← ET            | EH ← IT            | Thick broken line |
|                    | EH ← IT            | IH ← ET            | Thin broken line  |

to distinguish each elution mode on the phase distribution diagram, stationary phase retention curves were designed as indicated in Table IB. This symbolic design of the retention curves visually separates the effects of the two forces acting on the spiral column; the Archimedean screw effects produced by the head-tail orientation are shown by the solid and broken curves while the gradient force effects are given by the thick and thin curves.

## RESULTS AND DISCUSSION

### *Phase distribution diagrams for the helical column*

The first series of experiments was performed with the combined flow-through coil planet centrifuge having a 20 cm revolutionary radius. The use of a set of holders measuring 5, 10, and 15 cm in diameter provided  $\beta$  values of 0.125, 0.25, and 0.375, respectively.

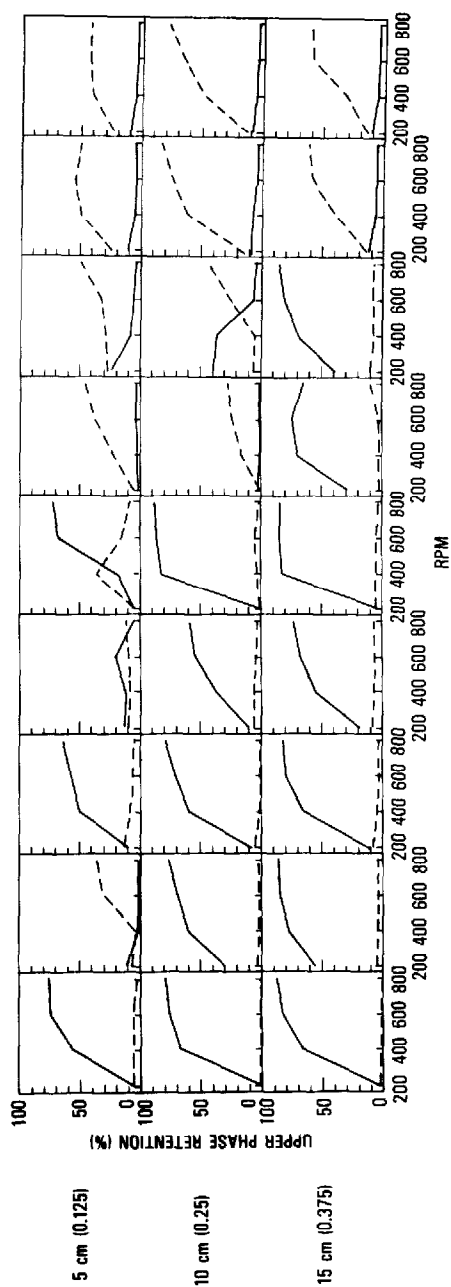
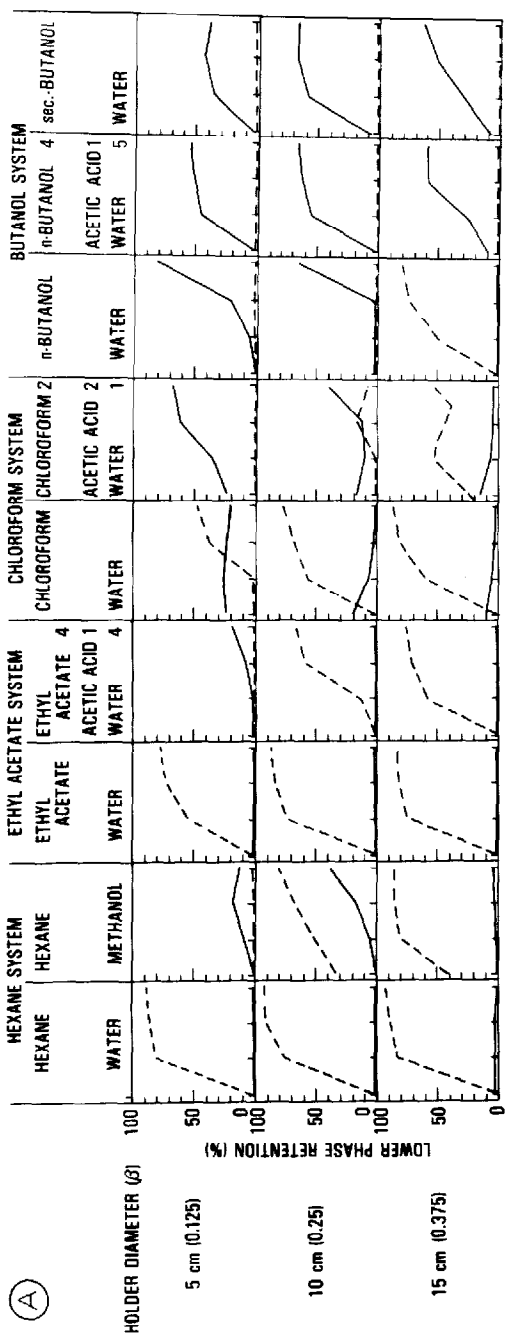
Fig. 2A illustrates a set of phase distribution diagrams obtained from the nine volatile solvent systems using the 1.6 mm I.D. helical column at three different  $\beta$  values. Both upper and lower phases were used as the mobile phase at a flow-rate of 120 ml/h except for the viscous butanol systems which were eluted at a reduced flow-rate of 60 ml/h. The upper block of diagrams was obtained with an upper mobile phase and the lower block, with a lower mobile phase. The middle rows of diagrams shown in each block were obtained using a 10 cm diameter holder ( $\beta = 0.25$ ) and are the same data illustrated in Fig. 4 (upper half) in Part I<sup>6</sup>.

Overall results clearly indicate that the phase distribution curves display diverse patterns according to the hydrophobicity of the solvent system and that within each solvent system the distribution pattern significantly changes with  $\beta$  values. As described in Part I, these solvent systems may be classified into three groups according to their characteristic retention profile in the phase distribution diagram.

The hydrophobic solvent group including hexane-water, ethyl acetate-water, and chloroform-water, displays high retention of the stationary phase if the upper phase is eluted in the tail to head mode (broken line) or the lower phase is eluted in the head to tail mode (solid line). The reversed elution modes result in almost no retention except for chloroform-water which produces 20–30% retention at a small  $\beta$  value of 0.125. This retention profile of the solvent group indicates that the upper phase has a strong hydrodynamic tendency to move toward the head and the lower phase toward the tail of the helical column regardless of the  $\beta$  values within the range investigated.

The hydrophilic solvent group represented by *n*-butanol-acetic acid-water (4:1:5) and *sec.*-butanol-water displays a retention profile contrasting with that of the hydrophobic solvent group. These solvent systems yield high retention of the stationary phase when eluting with the upper phase in the head to tail mode (solid line) or the lower phase in the tail to head mode (broken line), while no retention occurs with the reversed modes of elution. This retention profile of the hydrophilic solvent systems implies a reversed hydrodynamic tendency with the lower phase moving toward the head and the upper phase toward the tail within the range of  $\beta$  values studied.

The rest of the solvent systems belong to an intermediate solvent group which is characterized by moderate hydrophobicity of the organic phase. These solvent



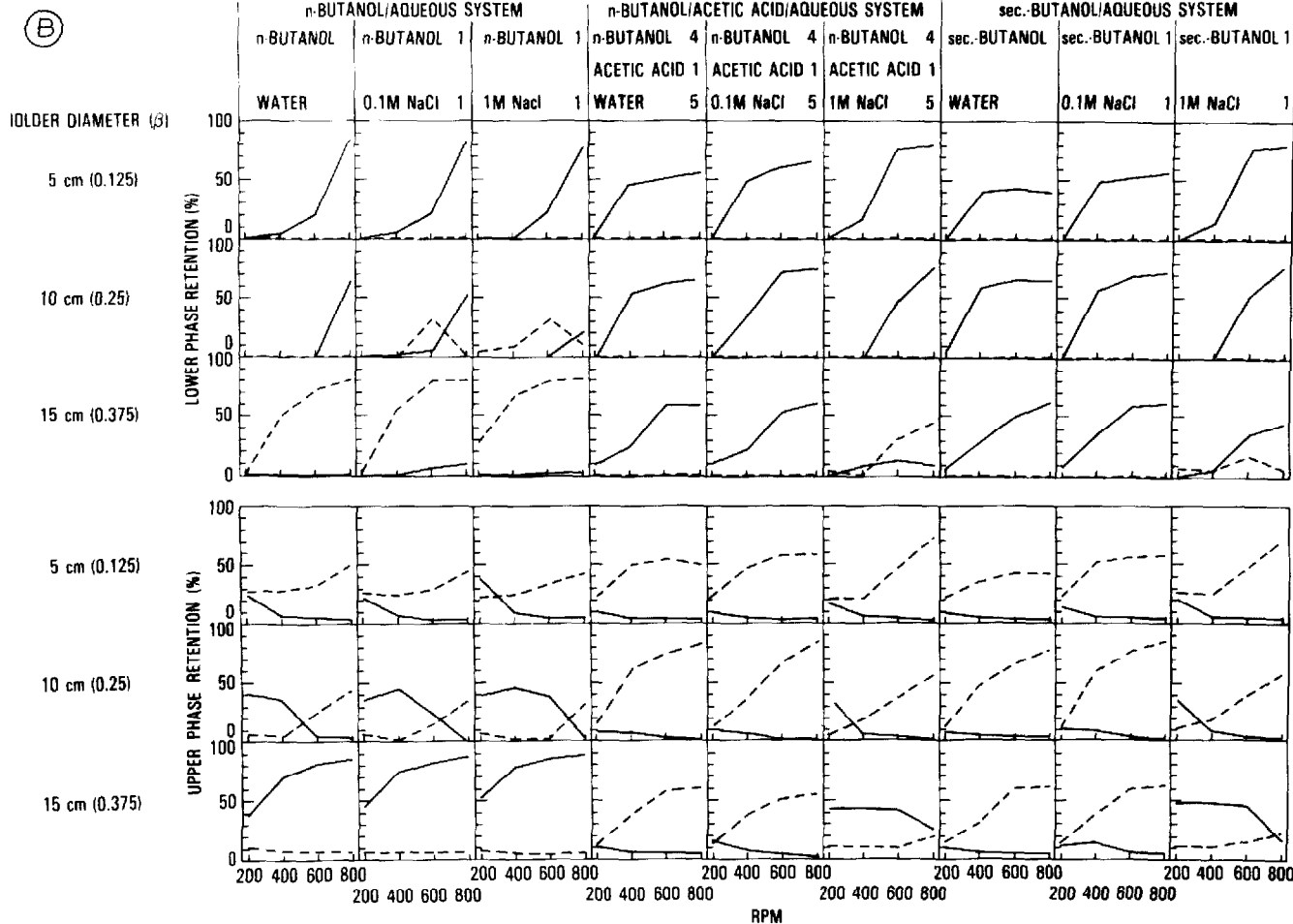


Fig. 2. Phase distribution in helical columns obtained under the Scheme IV planetary motion with a 20 cm revolutional radius. (A) Phase distribution diagrams of nine volatile solvent systems. (B) Phase distribution diagrams of butanol solvent systems. Experimental conditions are as follows: Planetary motion: Scheme IV (see Fig. 2 in Part I<sup>6</sup>). Revolutional speed: 200 to 800 rpm. Revolutional radius: 20 cm. Holder diameter ( $\beta$ ): 5 cm (0.125), 10 cm (0.25), 15 cm (0.375). Column: 1.6 mm I.D. single-layer helix. Flow-rate: 120 ml/h for hexane, ethyl acetate and chloroform systems and 60 ml/h for butanol systems. Mobile phase: upper phase (top three rows) and lower phase (bottom three rows). Elution mode: head to tail, solid line; tail to head, broken line (see Table IA).

systems exhibit a very complex retention profile which is sensitively affected by a change of the  $\beta$  value. At a small  $\beta$  value of 0.125, better retention of the lower phase is obtained under conditions represented by the solid curve while the broken curve indicates better retention of the upper phases. This pattern is analogous to that observed in the hydrophilic solvent group and indicates that the upper phase moves toward the tail and the lower phase toward the head under these conditions. At a larger  $\beta$  value of 0.375, this retention profile is completely reversed, *i.e.*, the broken curve shows greater lower phase retention and the solid curve shows greater upper phase retention analogous to the hydrophobic solvent group indicating that the upper phase moves toward the head and the lower phase, toward the tail. In brief, the hydrodynamic motion and resulting retention profiles of the intermediate solvent group approach those of the hydrophilic solvent group at small  $\beta$  value and become quite similar to those of the hydrophobic solvent group at large  $\beta$  value.

The butanol solvent systems were further modified by adding sodium chloride to study the effects of salt concentration on the retention curves under otherwise identical experimental conditions. The results of the experiments are summarized in Fig. 2B in a similar fashion. The phase distribution diagrams of *n*-butanol-water, *n*-butanol-acetic acid-water (4:1:5), and *sec.*-butanol-water which appeared on Fig. 2A are repeated in Fig. 2B for comparison. Addition of 0.1 *M* NaCl to the solvent system produces no significant effect on the retention curves in all solvent systems. However, at the 1 *M* NaCl concentration some interesting changes appear in the retention curves of the hydrophilic solvent systems. In both *n*-butanol-acetic acid-1 *M* NaCl (4:1:5) and *sec.*-butanol-1 *M* NaCl (1:1), increase in  $\beta$  value tends to depress the solid curve for the lower phase retention (top) and the broken curve for the upper phase retention (bottom) as if the whole curves are shifted toward the right. At  $\beta = 0.375$ , the other curves start to rise suggesting that, as the  $\beta$  value and/or the salt concentration are further increased, the retention profile of these solvent systems would become similar to those in the *n*-butanol-aqueous systems given at  $\beta = 0.375$ .

The studies on the effects of the  $\beta$  values were continued with the same set of helical columns using the second apparatus which produces Scheme IV planetary motion with a smaller revolutional radius of 10 cm, hence doubling the  $\beta$  value for each column. In order to compensate for the reduced revolutional radius, the experiments were performed at higher revolutional speeds ranging between 400 and 1000 rpm while other operational conditions were unaltered. The phase distribution diagrams obtained from this series of experiments are illustrated in Fig. 3A and B using the same format as in Fig. 2A and B to facilitate comparison.

Fig. 3A shows the phase distribution diagrams for the set of nine volatile solvent systems obtained from the helical columns rotated at a 10-cm revolutional radius. Compared with a 20-cm revolutional radius (Fig. 2A), both hydrophobic and intermediate solvent groups show substantial improvement in stationary phase retention, especially for the systems chloroform-acetic acid-water (2:2:1) and *n*-butanol-water. In these two solvent systems, the relative heights of the two retention curves in each diagram become reversed at the 10 cm helical diameter ( $\beta = 0.5$ ), while a similar reversal of the two curves occurred at the 15 cm helical diameter ( $\beta = 0.375$ ) with the 20 cm revolutional radius. In the hydrophilic solvent group, the retention of both *n*-butanol-acetic acid-water (4:1:5) and *sec.*-butanol-water was improved at the 5-cm helical diameter but it declined at the 10 to 15 cm helical diameter

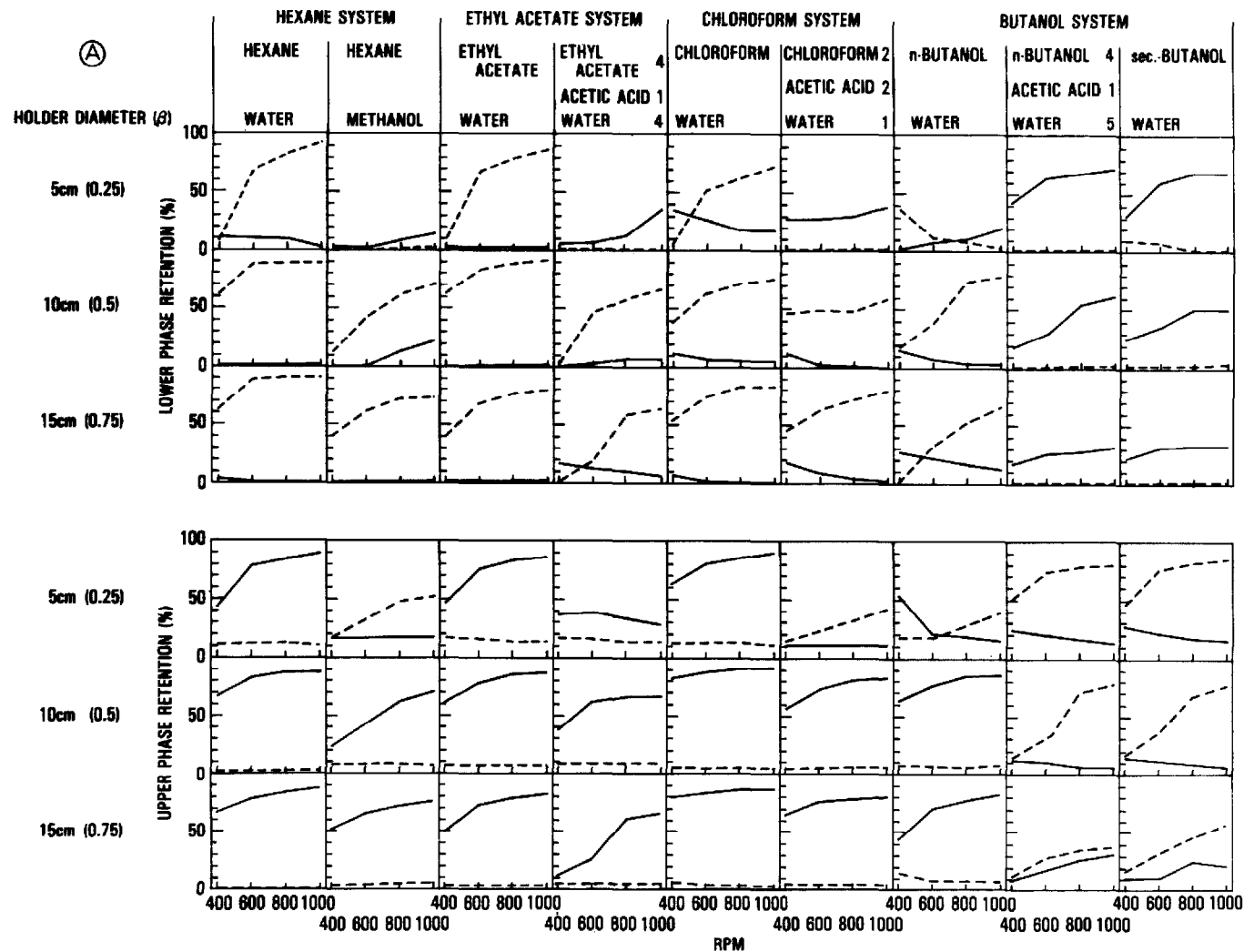


compared with the data obtained at the 20-cm revolutionary radius. Although there is no reversal of the two retention curves observed in the hydrophilic solvent group, the retention diagrams of the upper phase obtained at 15 cm holder diameter ( $\beta = 0.75$ ) show substantial rise of the solid curve which indicates that the reversal of the two curves might occur at a larger helical diameter.

Fig. 3B shows a similar set of diagrams for the butanol solvent systems obtained at the 10-cm revolutionary radius. Here again, the retention data of *n*-butanol-water, *n*-butanol-acetic acid-water (4:1:5), and *sec.*-butanol-water illustrated in Fig. 3A are repeated in Fig. 3B for comparison. Comparison of Figs. 2B and 3B shows some important effects of the altered revolutionary radius on the retention curves in all solvent groups. In the relatively hydrophobic *n*-butanol-aqueous systems, a 10-cm holder diameter at the 10-cm revolutionary radius gives the highest retention for all NaCl concentrations. All these systems show reversal of the retention curves between 5 and 10 cm helical diameters while with the 20-cm revolutionary radius a similar reversal of the retention curves occurred between the 10- and 15-cm helical diameters. In the hydrophilic solvent group of *n*-butanol-acetic acid-aqueous solution (4:1:5) and *sec.*-butanol-aqueous solution (1:1), 1 *M* NaCl concentration produces a complete reversal of the retention curves between 5 and 10 cm helical diameters and at the 15-cm helical diameter the retention profile becomes similar to those of the *n*-butanol-aqueous systems.

Phase distribution diagrams obtained from the helical columns under various experimental conditions (Figs. 2 and 3) show complex patterns of the retention curves which are, at present, unpredictable on a theoretical basis. The retention profile of the two-phase solvent system is certainly based on the hydrodynamic interaction of the two solvent phases in the rotating coiled column. As described earlier, for Scheme IV planetary motion the two solvent phases exhibit unilateral distribution along the axis of the coiled column in such a way that one phase entirely occupies the head side and the other phase, the tail side. Which phase is directed toward the head of the column is determined by the combined effects of various factors characteristic of the particular experimental condition. Overall results of the present studies suggest that conditions favoring upper phase movement toward the head and the lower phase toward the tail include: (1) increase in hydrophobicity of the nonaqueous phase; (2) increase in interfacial tension between the two solvent phases; (3) decrease in viscosity of the solvent phases; (4) increase in the density difference between the solvent phases; (5) increase in  $\beta$  value; (6) decrease in revolutionary radius; and (7) increase in the helical diameter of the column.

The effects produced by the physical properties of the solvent system (1-4), are intermingled and difficult to isolate from each other and, in addition, the effects of solvent-wall interaction should be further investigated with a column which provides a hydrophilic surface. The effects of the column factors (5-7), are also complex, but the  $\beta$  value may be the most sensitive parameter of the three. The revolutionary speed produces highly complex effects on the interaction of the two solvent phases, often causing a reversal of the unilateral hydrodynamic motion of the two phases which occurs at the crossing point of the two retention curves in the phase distribution diagram. In most cases increasing the revolutionary speed tends to direct the upper phase toward the head of the coiled column, *i.e.*, for lower phase retention, the broken curve crosses upward over the solid curve and for upper phase retention, the



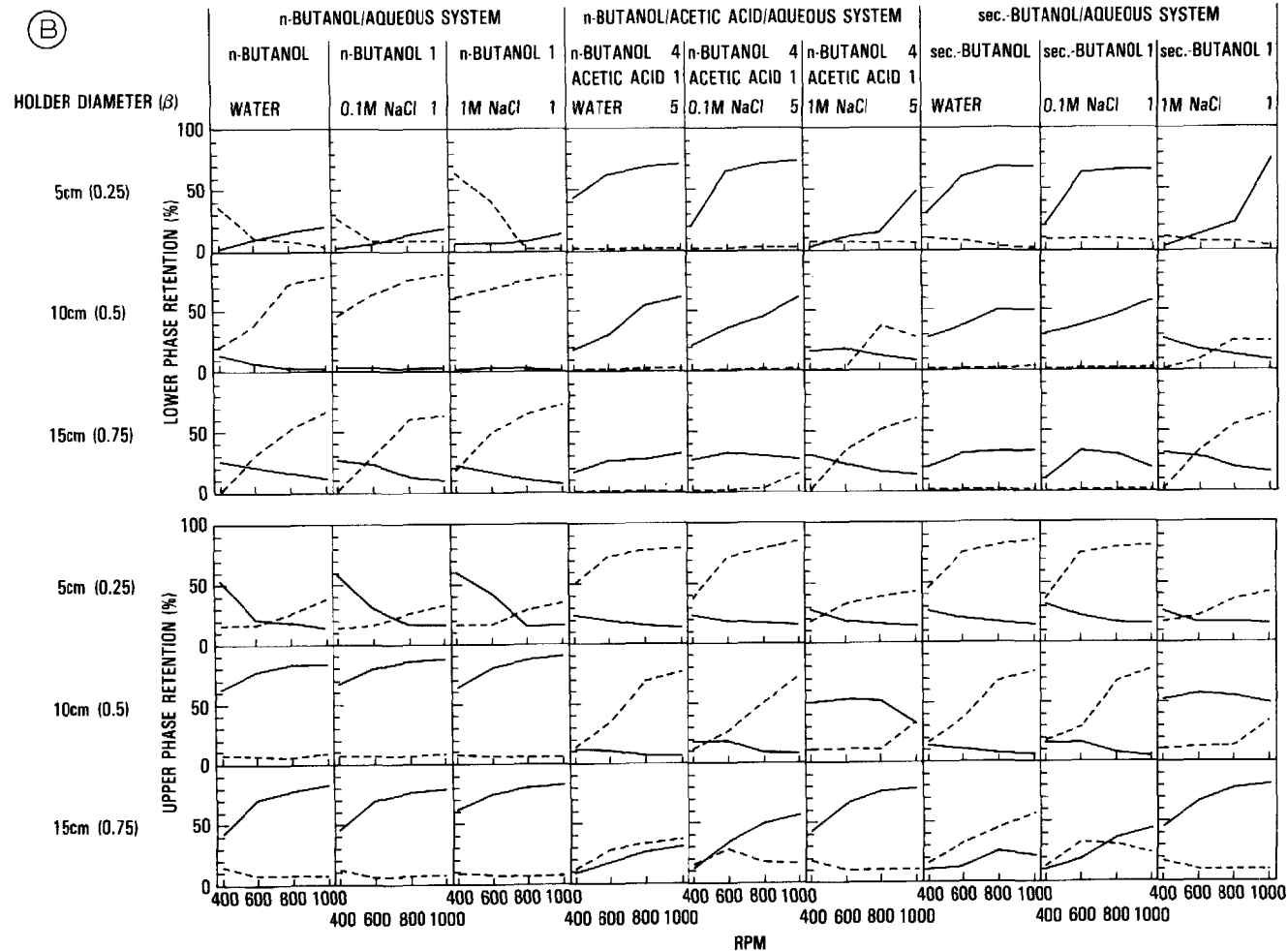
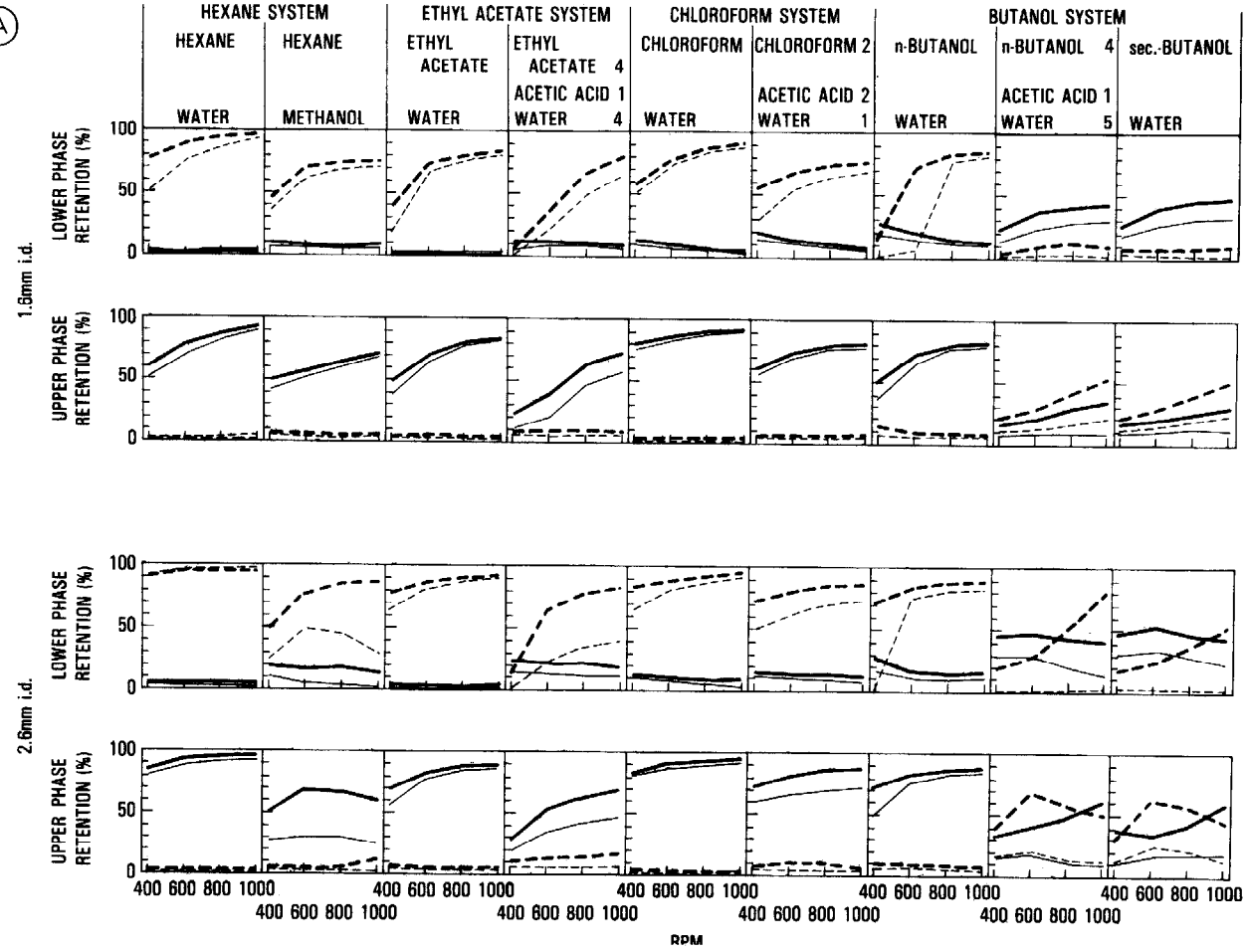


Fig. 3. Phase distribution in helical columns obtained under the Scheme IV planetary motion with a 10-cm revolutionary radius. (A) Phase distribution diagrams of nine volatile solvent systems. (B) Phase distribution diagrams of butanol solvent systems. Experimental conditions are as follows: Planetary motion: Scheme IV (see Fig. 2 in Part I<sup>o</sup>). Revolutinal speed: 400 to 1000 rpm. Revolutinal radius: 10 cm. Holder diameter ( $\beta$ ): 5 cm (0.25), 10 cm (0.5), 15 cm (0.75). Column: 1.6 mm I.D. single-layer helix. Flow-rate: 120 ml/h for hexane, ethyl acetate, and chloroform systems and 60 ml/h for butanol systems. Mobile phase: upper phase (top three rows) and lower phase (bottom three rows). Elution mode: head to tail, solid line; tail to head, broken line (see Table IA).

(A)

SINGLE-LAYER SPIRAL

$\beta = 0.5 - 0.8$  with 10 cm revolutional radius



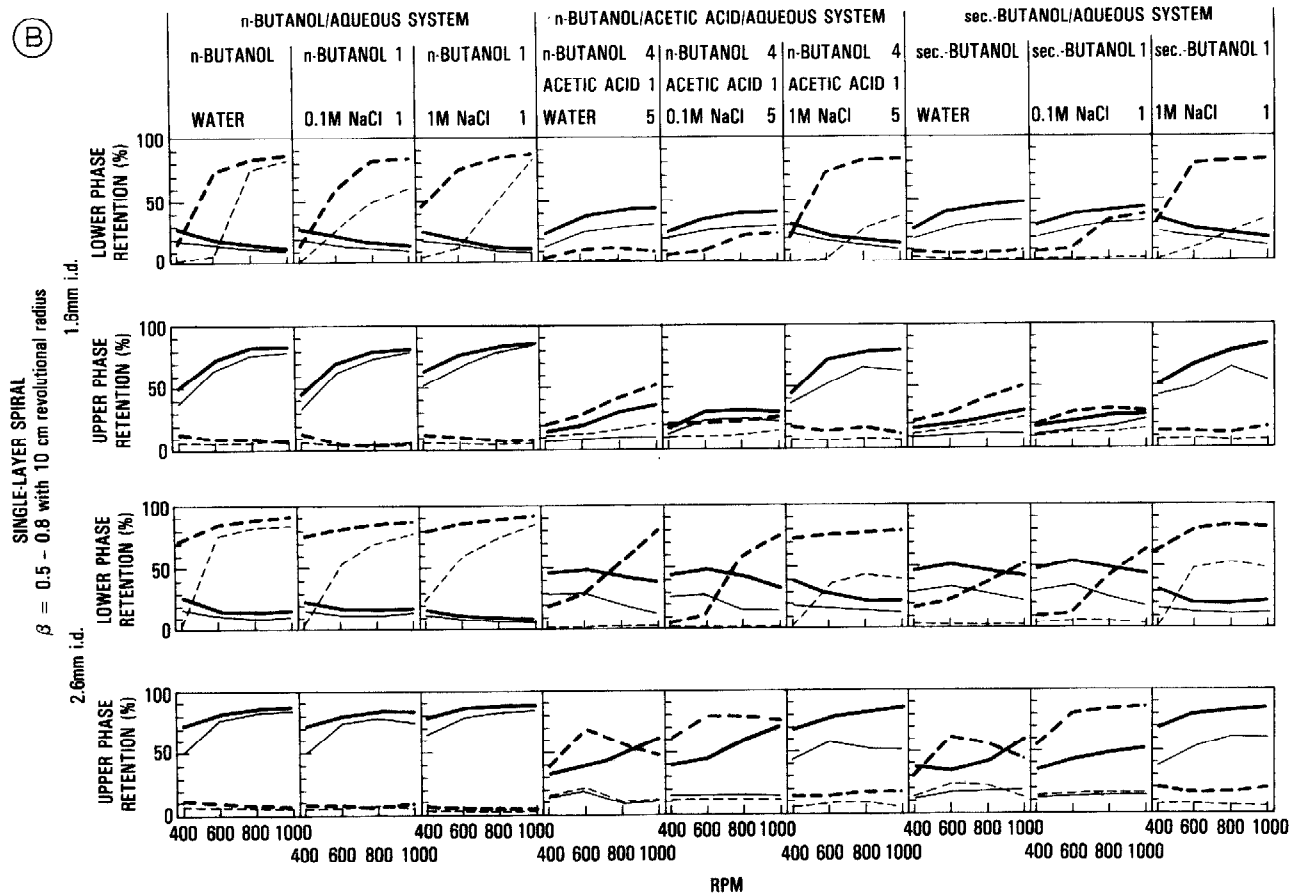


Fig. 4. Phase distribution in spiral columns obtained under the Scheme IV planetary motion with 10 cm revolutionary radius. (A) Phase distribution diagrams of nine volatile solvent systems. (B) Phase distribution diagrams of butanol solvent systems. Experimental conditions are as follows: Planetary motion: Scheme IV (see Fig. 2 in Part I). Revolutionary speed: 400 to 1000 rpm. Holder diameter ( $\beta$ ): 10 cm [0.5 (for the internal terminal)]-0.8 (for the external terminal)]. Column: 1.6 mm I.D. (2.4 mm O.D.) and 2.6 mm I.D. (3.6 mm O.D.) single layer spiral. Flow-rate: 120 ml/h for hexane, ethyl acetate and chloroform systems and 60 ml/h for butanol systems in 1.6 mm I.D. column (top two rows); the above rates are doubled for each solvent group in 2.6 mm I.D. column (bottom two rows). Mobile phase: upper phase (first row) and lower phase (second row) in each column. Elution mode: see Table IB.

solid curve crosses over the broken curve. However, reversal of the retention curves is also occasionally observed with butanol solvent systems. Although the flow-rate of the mobile phase is an important factor affecting the retention of the stationary phase, it is not likely to cause reversal of the unilateral hydrodynamic motion.

Among the various factors mentioned above, the  $\beta$  value may play a universal role in the unilateral hydrodynamic distribution of the two solvent phases. The phase distribution diagrams obtained at various  $\beta$  values strongly suggest that further increase of the  $\beta$  value by the use of a larger diameter holder would eventually reverse the direction of the unilateral hydrodynamic motion of the hydrophilic solvent systems. The decrease of the  $\beta$  value by the use of a smaller diameter holder may reverse the unilateral hydrodynamic motion of the hydrophobic solvent systems, as illustrated by the retention curves of chloroform-water at  $\beta = 0.125$  in Fig. 2A.

#### *Phase distribution diagrams obtained with spiral column*

A series of experiments has been performed to study the phase distribution in the spiral columns subjected to the Scheme IV synchronous planetary motion. In the spiral column retention of the stationary phase is affected by two forces; one is the Archimedean screw force governed by the head-tail relationship as in the helical column and the other, the centrifugal force field gradient created by the spiral pitch of the column.

As described earlier, the separate effects of these two forces are graphically presented in the phase distribution diagrams by the choice of symbols for drawing the retention curves as indicated in Table IB. The Archimedean screw effects are shown by the difference between the solid and broken curves as in the previous diagrams for the helical column while the centrifugal force gradient effects are indicated by the difference between the thick and thin curves. For simplification the choice of stationary and mobile phase giving the greater stationary phase retention as a result of the force gradient is shown by the thick line and the reversed elution mode by the thin line so that the thick curve always appears higher in the diagram regardless of the choice of the lower or upper layer as the mobile phase.

The phase distribution diagrams for the nine volatile solvent systems for single-layer spiral columns are illustrated in Fig. 4A where the top two rows show the data for the 1.6 mm I.D. column and the bottom two rows, the data for the 2.6 mm I.D. column. In order to provide similar linear-flow velocity of the mobile phase in each of the two columns, the flow-rate for the 2.6 mm I.D. column was made double that of the smaller column, *i.e.*, 240 ml/h for the hexane, ethyl acetate, and chloroform systems and 120 ml/h for the butanol systems.

The effects of the centrifugal force gradient in the spiral column on the retention curves are quite different in each solvent group. In the hydrophobic solvent group including hexane-water, ethyl acetate-water, and chloroform-water, the force gradient contributes little effect in either the 1.6 or 2.6 mm I.D. column as indicated by the small difference in retention level between the thick and thin curves for each pair of the solid and broken curves. In the intermediate solvent group including hexane-methanol, ethyl acetate-acetic acid-water (4:1:4), chloroform-acetic acid-water (2:2:1), and *n*-butanol-water, the effects of the force gradient becomes significant especially in the 2.6 mm I.D. column where much wider gaps were formed between the thick and thin curves. In the hydrophilic solvent group of *n*-butanol-

acetic acid–water (4:1:5) and *sec.*-butanol–water (1:1), the force gradient effects become further enhanced and except for the top diagrams (lower phase retention in the 1.6 mm I.D. column) both thick curves are higher than the pair of thin curves indicating that the force gradient plays a more important role in retention than the head–tail relation. In the 2.6 mm I.D. column, both hydrophilic solvent systems show a radical change in retention curves at the revolutionary speeds between 700 and 900 rpm where the two thick curves cross each other indicating that at higher revolutionary speeds the upper phase is directed toward the head and the lower phase toward the tail in the spiral column as is the case with other solvent groups.

Fig. 4B shows sets of phase distribution diagrams of butanol solvent systems obtained from the 1.6 mm I.D. and 2.6 mm I.D. spiral columns. Addition of NaCl to *n*-butanol–water produces no significant change in the stationary phase retention profile regardless of the internal diameter of the column. However, the retention curves for the *n*-butanol–acetic acid–water (4:1:5) and *sec.*-butanol–water are significantly altered at the 1 *M* NaCl concentration where the phase distribution profile becomes similar to those for the *n*-butanol–water and other intermediate solvent systems.

Performance of the spiral columns has also been examined with the third type of the coil planet centrifuge which provides greater  $\beta$  values by further reducing the revolutionary radius and/or increasing the holder diameter. The retention data were obtained from a 1.6 mm I.D. spiral column wound around a 15 cm diameter holder which was placed at 10 or 5 cm from the central axis of the centrifuge, providing the  $\beta$  values of 0.75–0.95 or 1.5–1.9, respectively. Fig. 5 shows a set of phase distribution diagrams obtained from four different solvent pairs with a 5 cm revolutionary radius (top two rows) and a 10 cm revolutionary radius (bottom two rows).

The retention data in Fig. 5 (bottom two rows) may be compared with those in Fig. 4A (the corresponding solvent systems in the top two rows) to observe the effects of increasing the holder diameter from 10 to 15 cm at the same revolutionary radius of 10 cm. The increase of the holder diameter substantially widens the gaps between the thick and the thin curves in intermediate solvent systems, indicating the enhanced force gradient effects on the retention of the stationary phase.

Comparison between the two top and the two bottom rows of the phase distribution diagrams in Fig. 5 shows the effects of reducing the revolutionary radius from 10 to 5 cm in the same spiral column on the 15-cm diameter holder. This reduction of the revolutionary radius not only causes an increase of  $\beta$  values but also halves the net centrifugal force field at a given revolutionary speed. The results clearly show that the retention levels are further decreased especially in the intermediate solvent systems, although widened gaps between the thick and thin curves in both hydrophobic and intermediate solvent systems indicate the enhanced effects of the force gradient on the retention of the stationary phase in the spiral column. The hydrophilic solvent system of *n*-butanol–acetic acid–water (4:1:5) shows no significant change when the revolutionary radius is reduced. Overall results indicate that the large  $\beta$  values in the spiral column obtained by increasing the holder diameter and decreasing the revolutionary radius produces substantial enhancement of the force gradient effects but fails to improve the retention of the stationary phase apparently due to the loss of the Archimedean screw effects produced by the head–tail hydrodynamic interaction of the two solvent phases.

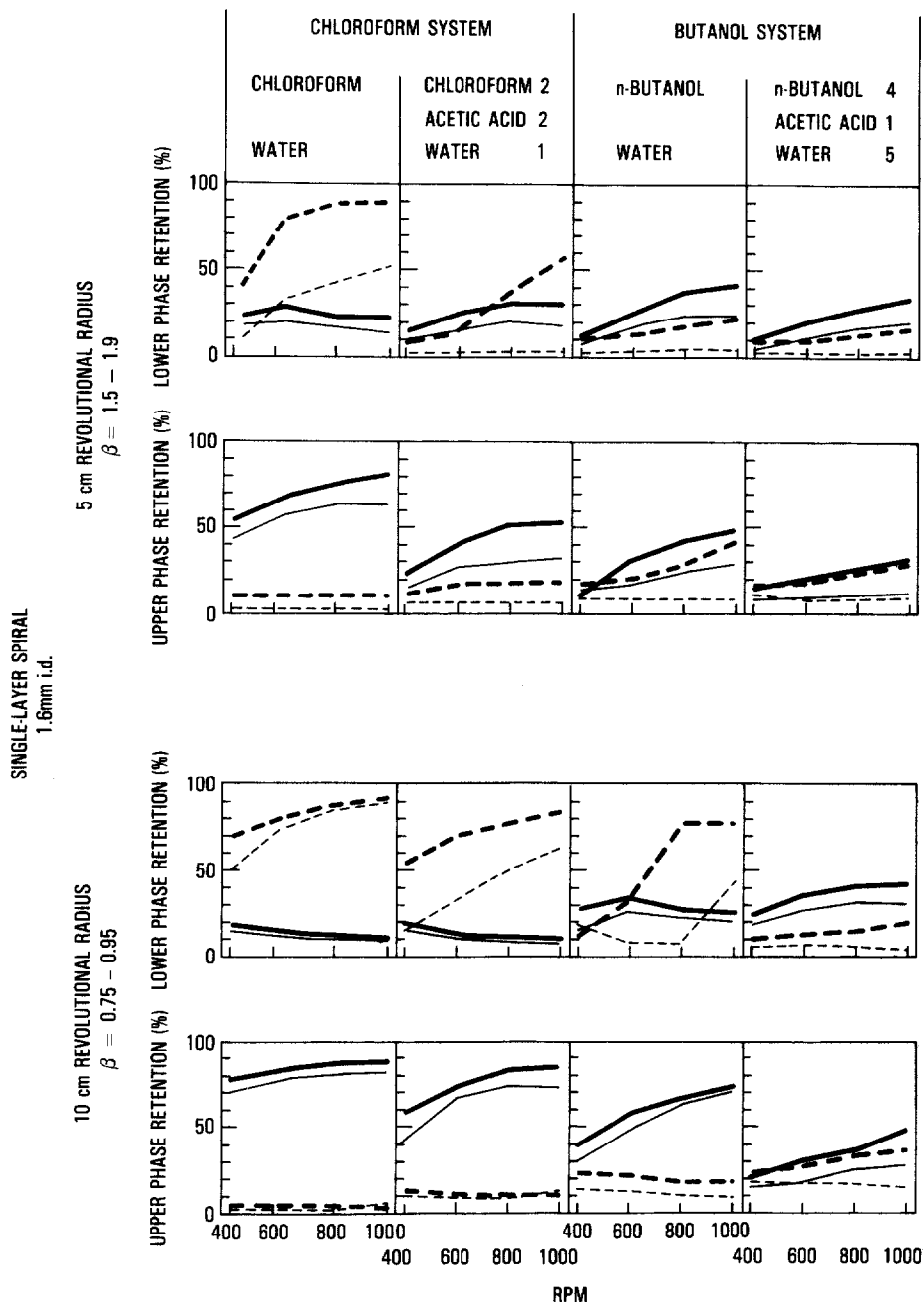


Fig. 5. Phase distribution in a spiral column obtained under the Scheme IV planetary motion with two different rotational radii. Experimental conditions are as follows: Planetary motion: Scheme IV (see Fig. 2 in Part I<sup>6</sup>). Rotational speed: 400 to 1000 rpm. Rotational radius: 5 cm (top two rows) and 10 cm (bottom two rows). Holder diameter ( $\beta$ ): 15 cm [1.5 (internal terminal)–1.9 (external terminal) and 0.75 (internal terminal)–0.95 (external terminal)]. Column: 1.6 mm I.D. (2.4 mm O.D.) single-layer spiral. Flow-rate: 120 ml/h for chloroform systems and 60 ml/h for butanol systems. Mobile phase: upper phase (first row) and lower phase (second row) in each group. Elution mode: see Table IB.



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