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RESEARCH ARTICLE

Two-phase Motion in Hydrodynamic Counter-current Chromatography

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ARTICLE HISTORY

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Abstract: ***Background***: Motion of the two mutually immiscible liquids in hydrodynamic counter-current chromatographic systems is speculated based on the observation of their behavior in a closed coiled tube rotating in unit gravity.

Materials and Methods: The experiment revealed an up and down pattern of four stages of two-phase volume ratio occupied at the head end of the coil according to the rotation speed. These two-phase behaviors are comprehensively explained on the bases of interplay between the unit gravity and centrifugal force generated by rotation of the coil. This theory is successfully extended to explain the two-phase behavior in a coil undergoing the type-I and type-J planetary motions.

Results and Discussion: The type-I planetary motion produces the centrifugal force distribution similar to that of slowly rotating coil in unit gravity (Stage I), where both phases competitively move toward the head of the coil. In contrast, the type-J planetary motion displays complex distribution patterns of centrifugal force according to the location of the coil on the holder hence the two-phase motion varies with the ß values. When ß is 0.5 – 0.75, the force pattern simulates that of the rotating coil in unit gravity at 120 rpm (Stage III) where the lighter phase moves toward the head leaving the heavier phase behind.

Conclusion: This clearly demonstrates the importance of the proper choice of ß values in high-speed countercurrent chromatography utilizing the type-J planetary motion.

Keywords: Archimedean screw effect, countercurrent chromatography, force distribution diagram, hydrodynamic CCC system, type-I planetary motion, type-J planetary motion.

1. INTRODUCTION

Countercurrent chromatography (CCC) utilizes two liquid phases in the column without the use of solid support [1-7]. The method is largely divided into two classes, *i.e.*, hydrostatic and hydrodynamic equilibrium systems. The hydrostatic system uses a stable force field to elute one of the phases as a mobile phase through a column filled with the other phase as the stationary phase [6, 8]. In this system, the stationary phase stays in the column as it is passively being displaced by the mobile phase, hence the motion of the two phases in the separation column is simple and easily understood. The hydrodynamic system, on the other hand, uses a rotating coiled column in either unit gravity or a centrifugal force field to generate an Archimedean screw effect which drives each phase competitively towards one end of the coil called the head, and the other end is called the tail [9-11]. This two-phase motion is clearly manifested by some of the hydrodynamic CCC systems such as a slowly rotating coil (5-10 rpm) in the unit gravity [12] and type-I synchronous planetary system [13], where two solvent phases in a closed coil competitively advance toward the head of the coil finally occupying the equal space in each coiled loop from the head towards the tail. However, in other systems, the two phases move in completely different ways. For example, in the coil rotated at 60 to 90 rpm under unit gravity, the heavier phase moves towards the head of the coil leaving the lighter phase behind at the tail end. In contrast, this hydrodynamic trend is completely reversed in the type-J planetary centrifuge, *i.e.*, the lighter phase advances toward the head of the coil leaving the heavier phase behind. In 1992, these strange behaviors of two immiscible phases in the rotating coiled tube have been explained in a paper entitled “Speculation on the mechanism of unilateral hydrodynamic distribution of two immiscible solvent phases in the rotating coil”. The paper was first submitted to the Science where the in-house review committee accepted it, but when the paper was sent out, it was rejected by one reviewer’s comment that the

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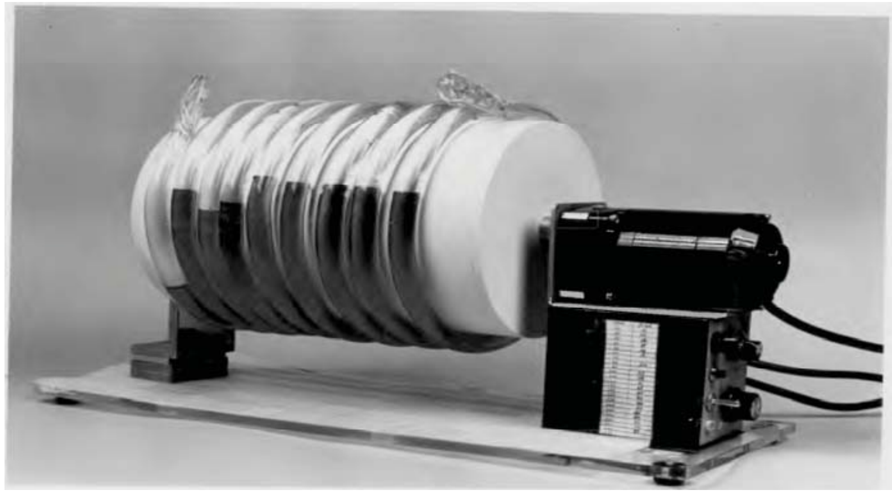

Fig. (1). Simple model of the rotating coil in unit gravity.

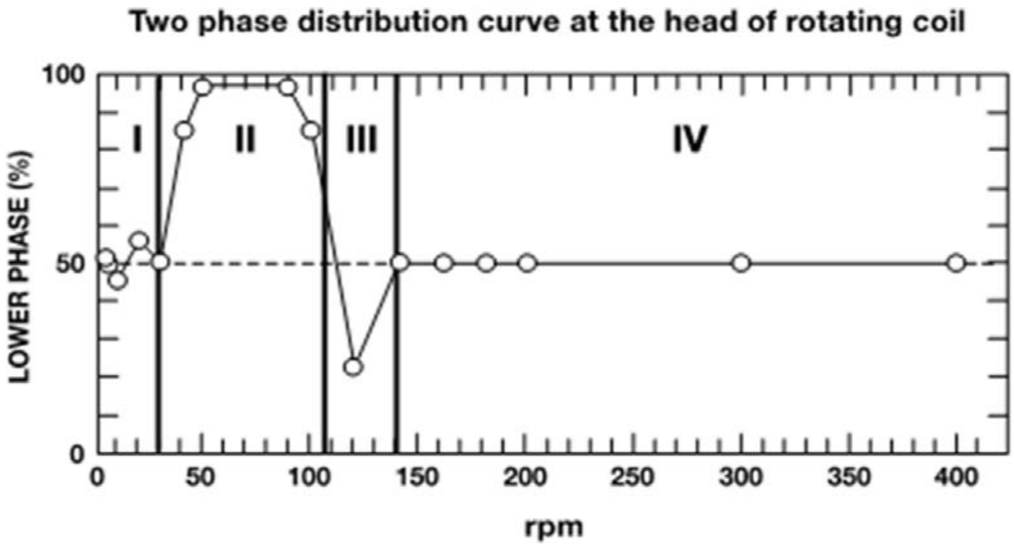

Fig. (2). Two-phase distribution curve of rotating coil in unit gravity.

paper uses 19th century mathematics (although in this 21st century, the mathematics used in the paper has not been improved yet). Consequently, the paper was published in the Journal of Liquid Chromatography [14]. Unfortunately, retrieving the article in this journal is highly charged and it is not easily available for most of the readers. Therefore, I decided to explain these hydrodynamic motions of the two phases more comprehensively in this article with the addition of new illustrations. This paper will answer the following questions:

1) Why does the heavier phase move towards the head of the coil in slow rotary CCC?

2) Why does the lighter phase move towards the head of the coil in high-speed CCC?

It should be noted that the two-phase motion in type J spiral column has been extensively studied by Sutherland *et al.* [15]. It has been believed that understanding these two phase motions in the hydrodynamic CCC systems are very important and will help readers further improve the design of the high-speed CCC systems.

This paper has been presented at the 9th International Conference on Countercurrent Chromatography: Ito, Y.: Two-phase motion in hydrodynamic countercurrent chromatography: Which phase travels towards the head of the coil?, The 9th International Conference on Countercurrent Chromatography, Chicago, IL, USA, August 1-3, 2016.

2. MATERIALS AND METHODS

2.1. Hydrodynamic Motion of the Two Phases in a Rotating Coil in Unit Gravity

A simple experiment using a rotating coil in the unit gravitational field will give an important hint to understand the two-phase motion in all the hydrodynamic CCC systems. Fig. (**1**) shows a simple model to study the hydrodynamic motion of the two phases enclosed in the rotating coil in unit gravity [14]. The glass coil is 20 cm in helical diameter and

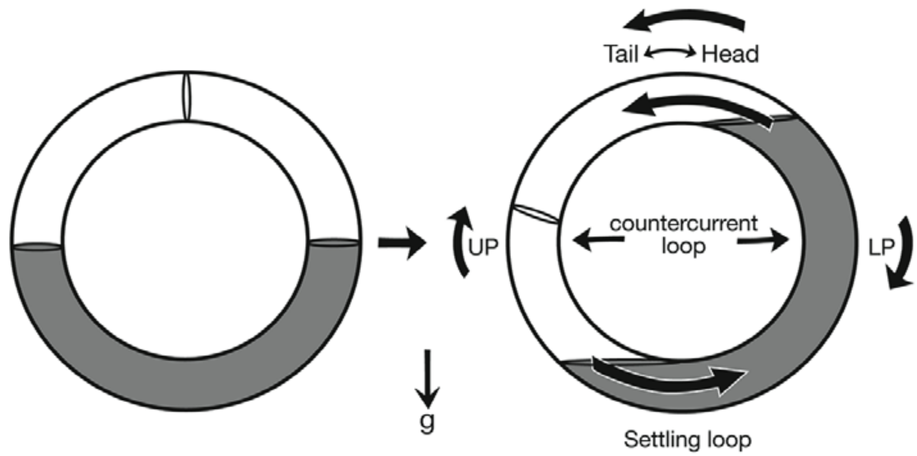

Fig. (3). Diagram of a portion of the coiled tube illustrates the hydrodynamic motion of the two phases in a rotating coiled tube in Fig. (**1**). The left stationary tube containing two solvent phases shows an interface in each lateral loop. The rotation of the tube as indicated by a curve arrow creates an Archimedean screw effect to form the head and tail orientation, and two interfaces move towards the tail as shown in the right diagram. The further rotation of the tube will result in overflow of the heavier phase towards the tail in the left loop and bubbling up of the lighter phase towards the tail on the right loop as shown by thick curved arrows in the tube. This causes the countercurrent movement of the lighter phase towards the head in the left loop and that of the heavier phase in the right loop of the tube as shown by short curved arrows.

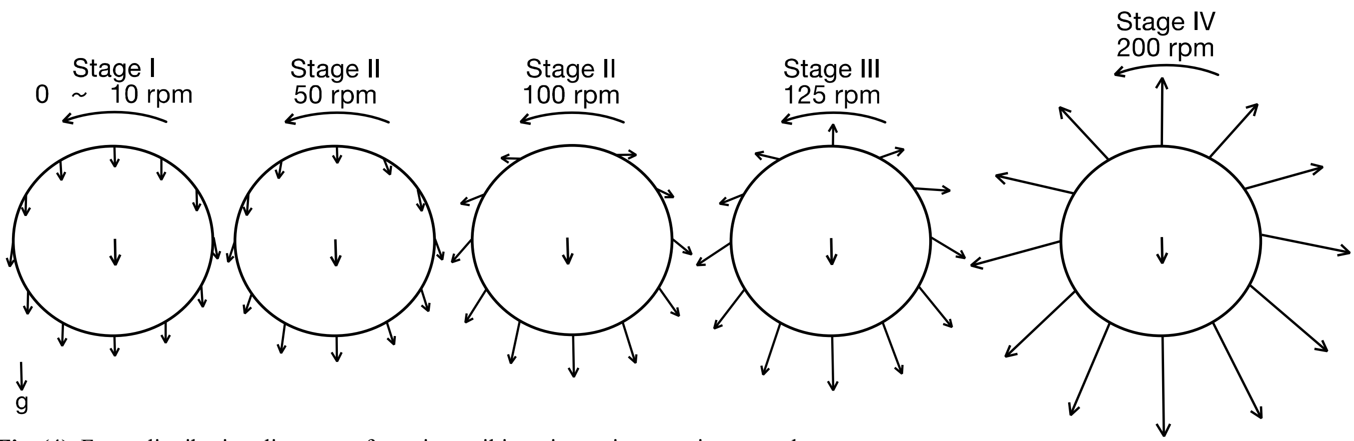

Fig. (4). Force distribution diagrams of rotating coil in unit gravity at various speeds.

5.5 mm in internal diameter, and it contains a two-phase solvent system composed of chloroform, acetic acid and 0.1M hydrochloric acid at a volume ratio of 2:2:1 at about equal volume of each phase. Sudan III was added to the solvent system to color the heavier chloroform phase. The experiment was performed to rotate the coil at a given speed until the two phases established hydrodynamic equilibrium. Then the rotation was stopped and the volume of each phase occupying at the head end of the coil was noted. The experiment was repeated by changing the rotation speed of the coil. The results of the experiment are shown in Fig. (**2**).

In this figure, the volume of the heavier phase occupying the head of the coil was plotted against the applied rotation speed in rpm. Percentage distribution of the heavier phase at the head of the coil shows an up-and-down curve which is divided into 4 stages as indicated in the figure. In Stage I at 0 – 10 rpm, two phases each occupy about equal volume at the head of the coil. As the rotation speed is increased in Stage II, the heavier phase tends to occupy more space in the head end and at 60 to 90 rpm, it completely occupies the head end of the coil. When the rotation speed is further increased to 120 rpm in Stage III, the lighter phase occupies more space at the head end, and at over 140 rpm, two phases again evenly occupy the head end each at 50 % in Stage IV. The formation of the above 4 Stages may be comprehensibly explained by interplay between the unit gravity and the centrifugal force generated by the rotation of the coil.

Fig. (**3**) schematically illustrates two-phase motion in a portion of the coiled tube shown in Fig. (**1**). When the stationary coiled loop containing an equal volume of each phase shown on the left is slowly rotated, the Archimedean screw effect creates the head and the tail of the coil as indicated at the top of coil as shown on the right diagram. When the coil rotation continues as indicated by the curved arrow, two phases undergo countercurrent movement through the lateral loops. In the left lateral loop, the heavier phase will continuously overflow downward through the loop toward the tail, and the lighter phase moves toward the head. This reveals an extremely important fact that the moving rate of the lighter phase towards the head is determined by the hydrodynamic condition or the acting force (the sum of the unit gravity and centrifugal force) at the top of the coil. If this

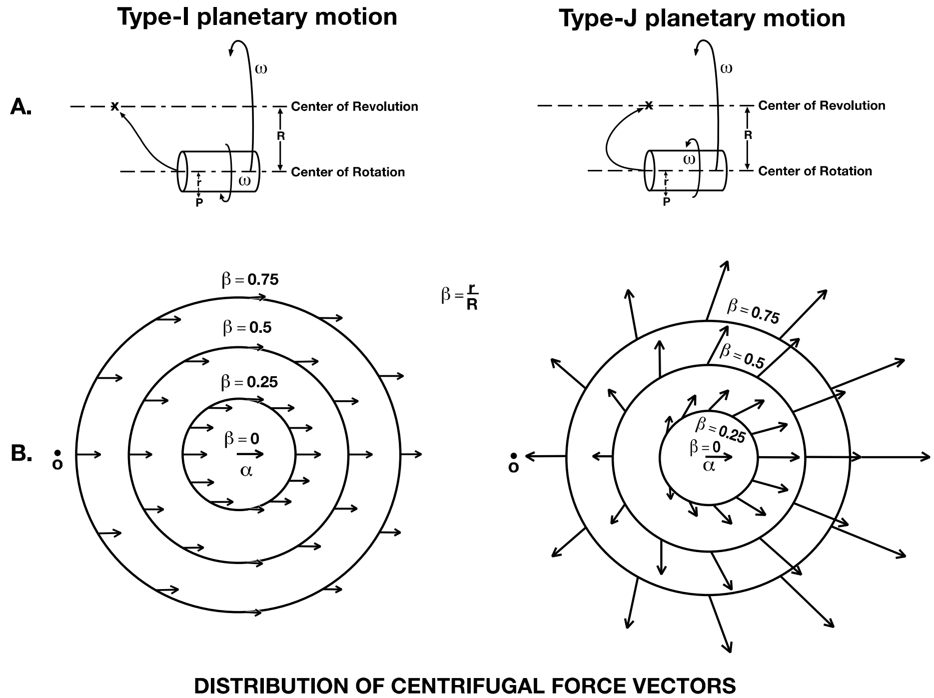

Fig. (5). (**A**, **B**) Planetary motion and the centrifugal force distribution diagrams of type-I and type-J coil plant centrifuges.

force is not strong enough to separate the two phases at the top of the coil, the lighter phase movement would be suppressed, and if the force is too strong to cancel the Archimedean screw effect, the lighter phase movement towards the head is again inhibited. In the right lateral loop, the lighter phase bubbles up through the heavier phase towards the tail while the heavier phase countercurrently moves toward the head of the coil. Similarly, heavier phase movement towards the head is determined by the hydrodynamic condition or the acting force at the bottom of the coil. Consequently, the two-phase motion toward the head of the coil is determined by the difference in sum of the unit gravity and the centrifugal force at between the top and the bottom of the coil. Therefore, formation of the four stages of the hydrodynamic curve in Fig. (**2**) may be explained by the force distribution diagrams shown in Fig. (**4**). At a low rotation speed of 0 – 10 rpm (Stage I) where the centrifugal force generated by rotation is negligible, the force acting at the top and the bottom of the loop is almost identical. Hence, the two phases competitively advance towards the head to occupy each 50% of the space at the head end of the coil. When the rotation speed is increased to 50-100 rpm (Stage II), the centrifugal force acting at the top and the bottom of the loop becomes stronger. Hence, the weakened force (gravity – centrifugal force) at the top delays the movement of the lighter phase on the left loop whereas the increased force (gravity + centrifugal force) at the bottom loop enhances the movement of the heavier phase toward the head. This results in 100% occupancy of the heavier phase at the head end. As the rotation speed is further increased to 125 rpm (Stage III), the top of the loop regains the force upward while too strong downward force at the lower portion of the loop inhibits the movement of the heavier phase through the right lateral loop. This results in enhanced movement of the lighter phase towards the head that causes the distribution curve crossing down the 50% line. When the rotation speed is further increased (Stage IV), strong centrifugal forces acting around the coil overcome the Archimedean screw force to distribute the lighter phase along the inner portion and the heavier phase along the outer portion throughout the coil. Therefore, each phase again occupies 50% at the head of the coil, since the equal volumes of the two phases are enclosed in the coil. The application of this system to CCC separation is most efficiently performed at the coil rotation between 50 and 100 rpm by pumping either the lighter phase from the head end or the heavier phase from the tail end of the rotating coil [16].

The above force distribution analysis can be further extended to the rotating coil in a centrifugal force field as described below.

2.2. Hydrodynamic Motion of the Two Phases in the Type-I Synchronous Planetary Motion

Fig. (**5A**) (top left diagram) diagrammatically shows the motion of a cylindrical column holder undergoing the type-I synchronous planetary motion. As indicated by curved arrows, the holder revolves around the central axis of the centrifuge while it rotates about its own axis in the opposite direction at the same angular velocity. This motion allows the flow tubes to rotate without twisting. The centrifugal force distribution diagram of this planetary motion is illustrated in Fig. (**5A**) (bottom left diagram). As clearly shown in the force diagram, every point on the holder is subjected to exactly the same centrifugal force which rotates around the column synchronously with the revolution. The force distribution diagram of this system closely resembles the rotating coil in unit gravity at a very low speed (Fig. **4**, Stage I, left), except that the unit gravity force is replaced with the centrifugal force. This clearly indicates that the two phases will competitively move towards the head of the coil in this system regardless of the rotation speed. Consequently, in this CCC system, separation should be done by pumping either phase from the head towards the tail of the coiled column while the retention of the stationary phase would be always less than 50% of the column capacity [13].

2.3. Hydrodynamic Motion of the Two Phases in the Type-J Synchronous Planetary Motion

The rotary-seal-free planetary motion of the type-J synchronous planetary centrifuge is shown in Fig. (**5B**) (top right diagram). The cylindrical column holder revolves around the central axis of the centrifuge while it synchronously rotates about its own axis in the same direction. The centrifugal force diagram produced by this planetary motion is shown in Fig. (**5B**) (bottom of right diagram). It shows a complex distribution of the force vectors around the coiled loop which is quite different from that in the type-I planetary motion. In this figure, four coaxial circles indicate location r on the coil at a different distance from the column axis where ß is r/R (R is distance between the column axis and the central axis of the centrifuge). The force distribution around the circle remarkably changes with ß values: At the ß value of 0.25, the distribution of centrifugal force vectors around the circle resembles the slow rotary system at Stage II (Fig. **4**, 100 rpm). This suggests that the lower phase would move towards the head of the coil. At the ß value of 0.5 – 0.75, the force vectors are all distributed outward while the force acting at the remote location becomes stronger resembling the slow rotary system at Stage III, which suggests that the lighter phase would dominantly move towards the head of the coil. In this high-speed CCC condition, the heavier phase should be pumped from the head to tail and the lighter phase from the tail to head in order to retain a large volume of the stationary phase in the column. With further increase of the ß value to over 1 (not shown in the figure), the force acting at the coil both near and remote from the center of revolution will become stronger to suppress the two-phase motion through the coil as in Stage IV in Figure 4 where the CCC application of this system would give low partition efficiency with a lack of the two-phase mixing in the coil.

3. RESULTS AND DISCUSSION

3.1. Speculation on the Motion of the Third Phase and its Application in Type-I CCC

The analysis of hydrodynamic motion of the two phases in the slowly rotating coil (Figs. **1** and **3**) will suggest some interesting applications for the separation of various samples. Let us consider the motion of the third phase such as solid, liquid (immiscible to either phase), or gas in the coil shown in Fig. (**3**).

3.2. Particles

When the particles, lighter than the heavier phase and heavier than the lighter phase, are present in the coil (Fig. **3**), they will distribute at or near the interface and move towards the tail in both lateral loops. Since the coil is rotating, they will stay in the left loop in a shorter period of time than in the right loop. Consequently, it is speculated that the larger and/or heavier particles with a higher sedimentation rate would delay movement towards the tail and therefore, the particles might be separated according to their size and/or density as described elsewhere [17].

3.3. Third Liquid Phase

Similarly to the motion of the particles stated above, the third liquid phase with moderate polarity and heavier than the lighter phase and lighter than the heavier phase present in the coil, will continuously move through the coil towards the tail. This hydrodynamic behavior of the third phase may be efficiently applied to CCC by pumping the third phase into the rotating coil filled with about equal volumes of the two phases. This will result in high retention of the two-phases in the coil and quickly elutes components with moderate polarity while other components with higher or lower polarities are retained longer in the coil. Using the third liquid phase for HSCCC has been reported by Shibusawa *et al.* [18].

3.4. Gas

When gas is present in a coil filled with liquid containing a surfactant, rotation produces foams which become the third phase since they are lighter than the liquid phase and the heavier than the gas phase. Consequently, the foam is expelled towards the tail of the rotating coil. Consequently, any compound which has an affinity to the foam will be quickly eluted with the foam [19]. This method could be also applied for separation of foaming compounds such as proteins and peptides without surfactant in the liquid [20]. These foam CCC methods reported earlier required some challenges in adjusting the eluting condition at a high rpm with a long coil due to high and fluctuating column pressure. This problem may be relieved by performing the separation using an end closed long coil containing about equal volumes of liquid and gas phases. The rotation will mix the gas and liquid to produce foams which will be constantly directed towards the tail. After some period of rotation, compounds with foam affinity accumulated at the tail end of the coil will be simply pushed out without rotating the coil.

CONCLUSION

Motion of two liquid phases enclosed in a rotating coil is described. The rotating coil in unit gravity shows 4 stages according to the rotation speed. Each stage was formed by the interplay between the unit gravity and centrifugal force generated by rotation. This theory is further extended to cover type-I and type-J planetary motions. It implies importance of choosing proper ß values for the coiled column in type-J HSCCC. The motion of the two phases also suggests some useful applications in high-speed CCC.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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Declared none.

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