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Comparison of the peak resolution and the stationary phase retention between the satellite and the planetary motions using the coil satellite centrifuge with counter-current chromatographic separation of 4-methylumbelliferyl sugar derivatives

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Abstract

Coil satellite centrifuge (CSC) produces the complex satellite motion consisting of the triplicate rotation of the coiled column around three axes including the sun axis (the angular velocity, ω_1), the planet axis (ω_2) and the satellite axis (the central axis of the column) (ω_3) according to the following formula: $\omega_1 = \omega_2 + \omega_3$. Improved peak resolution in the separation of 4-methylumbelliferyl sugar derivatives was achieved using the conventional multilayer coiled columns with ethyl acetate/1-butanol/water (3 : 2 : 5, v/v) for the lower mobile phase at the combination of the rotation speeds ($\omega_1, \omega_2, \omega_3$) = (300, 150, 150 rpm), and (1 : 4 : 5, v/v) for the upper mobile phase at (300 : 100 : 200 rpm).

The effect of the satellite motion on the peak resolution and the stationary phase retention was evaluated by each CSC separation with the different rotation speeds of ω_2 and ω_3 under the constant revolution speed at $\omega_1 = 300$ rpm. With the lower mobile phase, almost constant peak resolution and stationary phase retention were yielded regardless of the change of ω_2 and ω_3 , while with the upper mobile phase these two values were sensitively varied according to the different combination of ω_2 and ω_3 . For example, when $\omega_2 = 147$ or 200 rpm is used, no stationary phase was retained in the coiled column while $\omega_2 = 150$ rpm could retain enough volume of stationary phase for separation. On the other hand, the combined rotation speeds at ($\omega_1, \omega_2, \omega_3$) = (300, 300, 0 rpm) or (300, 0, 300 rpm) produced insufficient peak resolution regardless

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Conflicts of Interest

The authors declare no conflict of interest.

of the choice of the mobile phase apparently due to the lack of rotation speed except at (300, 0, 300 rpm) with the upper mobile phase. At lower rotation speed of $\omega_1 = 300$ rpm, better peak resolution and stationary phase retention were obtained by the satellite motion (ω_3) than by the planetary motion (ω_2), or $\omega_3 > \omega_2$.

The effect of the hydrophobicity of the two-phase solvent systems on the stationary phase retention was further examined using the *n*-hexane/ethyl acetate/1-butanol/methanol/water system at different volume ratios. In the satellite motion at $(\omega_1, \omega_2, \omega_3) = (300, 150, 150)$ rpm, almost constant stationary phase retention was obtained with the lower mobile phase regardless of the hydrophobicity of the solvent system whereas the stationary phase retention varied according to the volume ratio of the two-phase solvent system for the upper mobile phase. However, stable stationary phase retention was observed with either phase used as the mobile phase.

In order to analyze the acceleration acting on the coiled column, an acceleration sensor was set on the column holder by displacing the multilayer column. The combination of the rotation speeds at (300, 100, 200 rpm) showed double loops in the acceleration track, whereas (300, 150, 150 rpm) showed a single loop, and all other combinations showed, complex tracks.

The overall results indicate that the satellite motion is seriously affected by the combination of rotation speeds and the hydrophobicity of the two-phase solvent system when the upper phase was used as the mobile phase for separation.

Keywords

Counter-current chromatography; Instrumentation; Coil satellite centrifuge; Partition efficiency; Separation of sugar derivatives

1. Introduction

High-speed counter-current chromatography (HSCCC) using the type-J multilayer coil planet centrifuge (type-J CCC) and the cross-axis coil planet centrifuge (cross-axis CCC), has been widely used for the separation of various chemical compounds in both analytical and preparative scales [1]. The effective HSCCC separation was performed by the planetary motion such that the coiled column revolving around the central axis while synchronously rotating about its own axis. In the type-J CCC, the rotation axis of the coiled column is parallel to the revolution axis (central axis) of the rotary frame, while in the cross-axis CCC the rotation axis is orthogonal to the revolution axis [2, 3]. Both HSCCC instruments employ the rotary seal free flow-through system to avoid twisting the flow tubes and achieve stable high-speed revolution of the rotating column without a risk of leakage and contamination. High peak resolution with a satisfactory level of the stationary phase retention in the HSCCC separation is achieved by highly frequent repetition of mixing and settling process in a coiled tube by the aid of an Archimedean screw force generated by the planetary motion. We thought that the partition efficiency on HSCCC may be further improved by modifying the planetary motion by adding another rotary axis (satellite).

Our previous studies revealed that this coil satellite centrifuge (CSC) first designed and fabricated in our laboratory was successfully applied to the CCC separation of 4-

methylumbelliferyl sugar derivatives with organic-aqueous two-phase solvent systems [4]. Fig. 1 shows the chemical structures of 4-methylumbelliferyl sugar derivatives separated in this study. In the present CSC instrument, the coiled column rotates in its own axis (satellite axis) at the angular velocity of ω_3 , and simultaneously revolves around the planet axis (the angular velocity, ω_2) and further revolves around the sun axis (the angular velocity, ω_1) of the apparatus. In order to avoid the twisting flow tubes in this triplicate motion named the satellite motion, it is required that the motion of the rotating column should satisfy the following formula: $\omega_1 = \omega_2 + \omega_3$. Fig. 2 illustrates the schematic drawing of the satellite and the planetary motions in the present CSC instrument. At the combination of rotation speeds ($\omega_1, \omega_2, \omega_3$) = (300, 0, 300 rpm) or (300, 300, 0 rpm), the coiled column undergoes the planetary motion each with different revolution radius. At the rotation speed of $\omega_2 < \omega_3$, the rotation of the coiled column becomes the satellite motion more active and, at $\omega_2 > \omega_3$, the rotation becomes the planetary motion more active. Hence the present CSC instrument can perform either satellite or planetary motion by adjusting rotation speeds between ω_2 and ω_3 . The combination of rotation speeds ($\omega_1, \omega_2, \omega_3$) = (300, 150, 150 rpm) achieved a sufficient separation of five 4-methylumbelliferyl sugar derivatives with the lower mobile phase using the ethyl acetate/1-butanol/water system at the volume ratio of (3 : 2 : 5, v/v) and three sugar derivatives with the upper mobile phase using the similar solvent system at the volume ratio of (1 : 4 : 5, v/v) in the left-handed multilayer coiled column. However, it was found that changing the rotation speeds of ω_2 and ω_3 produced different stationary phase retention with the upper mobile phase, whereas other conditions were similar in these separations at a given ω_2 . The combination of rotation speeds ($\omega_1, \omega_2, \omega_3$) = (300, 147, 153 rpm) did not retain the lower stationary phase in the rotating column. In addition, the combination of rotation speeds (300, 100, 200 rpm) was not mechanically set due to the stress to the apparatus in counter clockwise rotation when using the column with a 75.0 mL capacity [4]. In the present study, improved peak resolution in the separation of 4-methylumbelliferyl sugar derivatives was achieved using the conventional multilayer coiled columns at different rotation speeds of ω_2 and ω_3 , and the effect of the satellite motion on the peak resolution and the stationary phase retention was compared with the planetary motion by replacing the side motor to more powerful one. The stationary phase retention is mainly affected by the hydrophobicity of the two-phase solvent system used for separation and the physical force acting on the rotating column. The two-phase solvent system with higher hydrophobicity generally retains the stationary phase with enough volume for separation. The effect of the hydrophobicity of the organic-aqueous two-phase solvent systems on the stationary phase retention was examined in both the satellite and the planetary motions. The acceleration produced by the satellite motion was analyzed by an acceleration sensor set on the column holder to reveal the reason why the drastic change of the stationary phase retention was observed when the lower phase was used as the stationary phase.

2. Experimental

2.1 Apparatus

The CSC instrument used in the present studies was fabricated at the Machining Technology Center of Nihon University (Chiba, Japan). The design of the CSC instrument has been described in detail elsewhere [4]. The original side motor (Type NX620AC-2, rated output

power: 200 W, Oriental Motor Co., Tokyo, Japan) used in the previous study [4] was replaced with the more powerful one (Type NX975AS-2, rated output power: 750 W, Oriental Motor Co.).

The acceleration sensor (Type SS-WS1103) and its accessory (Type SS-WSEK01) were purchased from Sports Sensing Co. (Fukuoka, Japan).

2.2 Preparation of the conventional multilayer coiled column assemblies

The left-handed multilayer coil used in the previous study [4] and the conventional multilayer coil (alternatingly left- and right-handed coil) employed in the present study are schematically illustrated in Fig. 3.

The conventional multilayer coil was prepared by winding a 1.5 mm I.D. and 2.5 mm O.D. PTFE (polytetrafluoroethylene) tubing (Flon Kogyo, Tokyo, Japan) around the holder hub of 3 cm diameter forming four coiled layers between a pair of flanges spaced 5 cm apart. Each coiled column was prepared as follows: The tubing was directly wound onto the holder hub starting on the top side. When the coil reached the bottom side, the layer was wrapped with an adhesive tape and the tubing was resumed winding the next layer from the bottom side to the top side in the same direction from the view of the top side. It results in a conventional multilayer coil assembly composed of alternating left- and right-handed coils, which is identical to that conventionally used in the type-J CCC. Four conventional multilayer coiled column assemblies were connected in series with flow tubes, where two identical coil assemblies were diagonally mounted on each second rotary frame that is symmetrically set around the planet axis. The total column capacity was 75.0 mL.

2.3 Reagents

4-Methylumbelliferyl sugar derivatives used as test samples were obtained as follows: 4-Methylumbelliferyl- α -L-fucopyranoside (α -L-Fuc), 4-methylumbelliferyl- β -D-fucopyranoside (β -D-Fuc), 4-methylumbelliferyl- β -D-glucopyranoside (Glc), 4-methylumbelliferyl- β -D-galactopyranoside (Gal) and 4-methylumbelliferyl- β -D-cellobioside (Cel) were purchased from BIOSYNTH AG (Staad, Switzerland). 4-Methylumbelliferyl- α -D-mannopyranoside (Man) was obtained from Sigma (St. Louis, MO, USA). All other reagents were of reagent grade.

2.4 Preparation of two-phase solvent system and sample solutions

The organic-aqueous two-phase solvent systems used in the present studies were composed of ethyl acetate/1-butanol/water (3 : 2 : 5, v/v) for the lower mobile phase and (1 : 4 : 5, v/v) for the upper mobile phase [4, 5]. The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated after two clear layers were formed.

The sample solution was prepared by dissolving the standard sample mixture with equal volumes of each phase of the two-phase solvent system used for separation.

2.5 CCC separation of 4-methylumbelliferyl sugar derivatives

Each separation was initiated by completely filling the column with the stationary phase, followed by injection of the sample solution (ca. 2 mL) into the column inlet with a syringe. After the column was rotated with the desired combination of the rotation speeds of ω_1 , ω_2 and ω_3 , the mobile phase was pumped into the column at a suitable flow rate in the head to tail elution mode using a reciprocating pump (Model KHU-W-52, Kyowa Seimitsu, Co., Tokyo, Japan). The rotation direction of each axis is counterclockwise (CCW) (sun axis) – CCW (planet axis) – CCW (satellite axis) for the lower mobile phase and clockwise (CW) (sun axis) – CW (planet axis) – CW (satellite axis) for the upper mobile phase. The effluent from the outlet of the column was fractionated into test tubes at 2 min/tube using a fraction collector (Model CHF 100AA, Advantec, Tokyo, Japan). The separating conditions, i.e., sample composition, flow rate and so on, were given in the figure captions.

2.6 Analysis of fractions

Each separated fraction of 4-methylumbelliferyl sugar derivatives was diluted with a desired constant volume of methanol and the absorbance was measured at 318 nm with a spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan).

2.7 Evaluation of partition efficiency

The partition efficiencies in each separation were evaluated by peak resolution (R_s) computed from the chromatogram according to the conventional equation and stationary phase retention measured after each separation [1].

3. Results and discussion

3.1 CCC separation of 4-methylumbelliferyl sugar derivatives with the common multilayer coiled columns in the satellite and the planetary motions

Our previous studies demonstrated that effective separation of 4-methylumbelliferyl sugar derivatives was achieved using the left-handed multilayer coiled columns in the satellite motion with the combination of the rotation speeds, $(\omega_1, \omega_2, \omega_3) = (300, 150, 150 \text{ rpm})$ [4]. In order to improve the peak resolution between these sugar derivatives, the CSC separation was further examined using the common multilayer coiled columns at various combinations of rotation speeds, ω_2 and ω_3 , under the constant revolution speed at $\omega_1 = 300 \text{ rpm}$. Fig. 4A illustrates the separation of 4-methylumbelliferyl sugar derivatives using the ethyl acetate/1-butanol/water system at the volume ratio (3 : 2 : 5, v/v). With the lower mobile phase, the peak resolution in the separation of five 4-methylumbelliferyl sugar derivatives including Cel, Glc, Man, β -D-Fuc and α -L-Fuc obtained by the conventional multilayer coiled columns at the combination of rotation speeds (300, 150, 150 rpm) was almost similar to those obtained by the left-handed multilayer coiled columns used in the previous studies [4]. With the upper mobile phase as illustrated in Fig. 4B, better peak resolution in the separation of three 4-methylumbelliferyl sugar derivatives including α -L-Fuc, Gal and Cel was obtained by the conventional multilayer coiled columns at the rotation speed combination, (300, 100, 200 rpm) than that obtained by the left-handed multilayer coiled columns at (300, 150, 150 rpm) [4].

Fig. 5 illustrates the CCC separation of 4-methylumbelliferyl sugar derivatives using the CSC at two different types of the column rotation. At the combination of the rotation speeds, (300, 300, 0 rpm), insufficient peak resolution between Man and β -D-Fuc was obtained with the lower mobile phase (Fig. 5A, left side) apparently due to low stationary phase retention (Fig. 6A, $\omega_2 = 300$ rpm). The separation failed with the upper mobile phase (Fig. 5B, left side) due to no retention of the stationary phase (Fig. 6B, right side, $\omega_2 = 300$ rpm). At the combination of the rotation speeds, (300, 0, 300 rpm), with the lower mobile phase insufficient peak resolution between Man and β -D-Fuc was also obtained (right upper side) although enough volume of the stationary phase was retained in the column (Fig. 6, left side, $\omega_2 = 0$ rpm) whereas with the upper mobile phase the baseline separation of broad peaks between three sugar derivatives was achieved (right lower side) with high retention of the stationary phase (Fig. 6, right side, $\omega_2 = 0$ rpm).

The overall results indicate that better peak resolution of sugar derivatives is produced by the satellite motion higher than the planetary motion ($\omega_3 > \omega_2$) at the low revolution speed ($\omega_1 = 300$ rpm).

3.2 Effect of the rotation speeds, ω_2 and ω_3 , on the stationary phase retention and the peak resolution in the satellite and the planetary motions

The effect of rotation speeds, ω_2 and ω_3 , on the stationary phase retention and the peak resolution was examined in detail by the separation of 4-methylumbelliferyl sugar derivatives with the ethyl acetate/1-butanol/water system at the volume ratio of (3 : 2 : 5, v/v) for the lower mobile phase and (1 : 4 : 5, v/v) for the upper mobile phase [4, 5]. With the lower mobile phase as illustrated in Fig. 6A, almost constant stationary phase retention and peak resolution were obtained at the rotation speed (ω_2) from 100 to 200 rpm. However, with the upper mobile phase as illustrated in Fig. 6B, both the stationary phase retention and the peak resolution were sensitively changed by the increase of the rotation speed, ω_2 , from 100 to 200 rpm, because slight change of ω_2 resulted in the drastic change of these two values. Especially, the rotation speeds at 147 and 200 rpm did not retain any stationary phase in these conventional multilayer coiled columns. The overall results indicate that the satellite motion produces the drastic change of the stationary phase retention and the peak resolution by varying the rotation speeds, ω_2 and ω_3 when the upper phase was used as the mobile phase.

3.3 Effect of the hydrophobicity of the n-hexane/ethyl acetate/1-butanol/methanol/water system on the stationary phase retention in the satellite and the planetary motions

As described above, the stationary phase retention with the upper mobile phase highly depended on the rotation speeds, ω_2 and ω_3 , while almost constant stationary phase retention was obtained with the lower mobile phase regardless of the change of the rotation speeds. In order to elucidate the reason of this contrasting difference, the effect of the hydrophobicity of the *n*-hexane/ethyl acetate/1-butanol/methanol/water system [6] on the stationary phase retention was examined using the combination of rotation speeds at (300, 150, 150 rpm) for the satellite motion and (300, 0, 300 rpm) for the planetary motion upon modifying the hydrophobicity of this solvent system by systematically changing the volume ratio of the solvent composition as summarized in Table 1. The solvent system No. 1 is most

hydrophobic and the solvent system No. 16 is most hydrophilic among these solvent systems. Fig. 7 illustrates the effect of the hydrophobicity of 16 solvent systems on the stationary phase retention produced by satellite and planetary motions. With the lower mobile phase (Fig. 7A), the satellite motion produced increased stationary phase retention in more hydrophobic solvent systems while the planetary motion yielded almost constant stationary phase retention regardless of the difference in hydrophobicity of the solvent systems. With the upper mobile phase (Fig. 7B), the satellite motion showed relatively low stationary phase retention with most of the solvent systems, and none at all with three solvent systems (Nos. 10 – 12), while the planetary motion showed relatively high stationary phase retention regardless of the difference in hydrophobicity of the solvent systems. As summarized in Table 1, the specific gravity of the upper phase and the lower phase of each two-phase solvent system was measured using a gravimeter at room temperature. The difference of the specific gravity between the upper and the lower phases closely correlates with the settling time of the two phases. Therefore, settling times of these 16 solvent systems reported by Oka *et al.* [6] were also summarized in Table 1. This result indicates that the solvent system with a small difference in the specific gravity requires long settling time. Almost similar tendency was obtained in the retention of the lower phase according to the difference in specific gravity between the two phases, except for very hydrophobic solvent systems of No. 1 – 4.

Fig. 8 illustrates the effect of the rotation speeds, ω_2 and ω_3 , on the stationary phase retention of four different solvent systems (No. 6, 7, 10 and 15). Regardless of the difference of the solvent systems, the rotation speeds (ω_2), 147 and 200 rpm, resulted in extremely low stationary phase retention when the upper phase was used as the mobile phase. The overall results indicate that the satellite motion with the upper mobile phase is sensitively affected by the difference in the hydrophobicity of the two-phase solvent systems as well as by the rotation speeds, ω_2 and ω_3 .

3.4 Acceleration change in the satellite motion at various combination of rotation speeds

In general, stationary phase retention is mainly affected by the interfacial tension of two-phase used for separation and the centrifugal forces (negative value to the acceleration) acting on the solvent system in the rotating column. As described above, regardless of the difference of the solvent systems, the rotation speeds at (300, 147, 153 rpm) and (300, 200, 100 rpm) produced extremely low stationary phase retention with the upper mobile phase. This result suggests that the physical forces acting on the solvent systems in the column at these particular combinations of rotation speeds may sensitively affect the stationary phase retention with the upper mobile phase. The acceleration pattern produced by the column rotation can be used to predict the satisfactory retention of the stationary phase in the column. In order to monitor the acceleration, the acceleration sensor was set on the column holder as illustrated in Fig. 9. The acceleration changes obtained by the acceleration sensor during a period of one minute with eight combinations of rotation speeds were shown in Fig. 10 for the CCW (ω_1) – CCW (ω_2) – CCW (ω_3) and in Fig. 11 for the CW (ω_1) – CW (ω_2) – CW (ω_3) rotating directions. Different acceleration changes were obtained in each combination of the rotation speed while similar acceleration pattern was obtained regardless of the difference of the directions. At (300, 150, 150 rpm) which produced better peak

resolution in the separation of sugar derivatives with the lower mobile phase, the single loop was found in the acceleration track. At (300, 100, 200 rpm) which produced improved peak resolution with the upper mobile phase, the double loop was found in the acceleration track. All other combinations except for (300, 200, 100 rpm) produced complex acceleration tracks. The combination (300, 200, 100 rpm) drew a double loop consisting of a single circle containing a small heart shaped acceleration track. This result suggests that the combination (300, 200, 100 rpm) does not retain the stationary phase due to the lack of revolution speed (ω_1), which is different from the case in no stationary phase retention at the combination (300, 147, 153 rpm). The combinations from (300, 100, 200 rpm) to (300, 147, 153 rpm) ($\omega_2 < \omega_3$) derive the satellite motion more than the planetary motion, whereas the combinations from (300, 153, 147 rpm) to (300, 200, 100 rpm) ($\omega_2 > \omega_3$) derive the planetary motion more than the satellite motion as illustrated in Fig. 2. At the combination (300, 150, 150 rpm), the satellite motion equals with the planetary motion ($\omega_2 = \omega_3$). As to the reason why the combination (300, 147, 153 rpm) does not retain the stationary phase, the response was not found from the acceleration monitoring because the difference of the acceleration tracks between the combination (300, 147, 153 rpm) and other combinations including (300, 135, 165 rpm), (300, 153, 147 rpm), (300, 160, 140 rpm) and (300, 165, 135 rpm) was not clear. However, gradual change of the acceleration track in each rotation may affect the stationary phase retention because the physical force acting on the two-phases in the column is changing with each rotation. A set of these experiments revealed that slight change of the combination of rotation speeds produced the acceleration diversity in the satellite motion. The overall results indicate that the combination of rotation speeds (300, 100, 200 rpm) may be suitable for either phase used as the mobile phase for CSC separation.

4. Conclusion

Improved peak resolution in the separation of 4-methylumbelliferyl sugar derivatives was achieved using the CSC instrument by replacing the left-handed multilayer coiled column with the conventional multilayer coiled columns. Better CSC separation and stationary phase retention was obtained by the combination of rotation speeds at ($\omega_1, \omega_2, \omega_3$) = (300, 150, 150 rpm) for the lower mobile phase and at (300, 100, 200 rpm) for the upper mobile phase. The retention of the lower phase was affected by the hydrophobicity of the organic-aqueous two-phase solvent system and the combination of rotation speeds. The analysis of the acceleration change in the satellite motion revealed that the acceleration track was sensitively altered by the combination of rotation speeds. The combination (300, 150, 150 rpm) forms a single loop and (300, 100, 200 rpm), a double loop in the acceleration track, respectively. At lower rotation speeds, better partition efficiencies were obtained by setting the satellite motion higher than the planetary motion. These results may provide a useful guidance for selecting the optimum experimental conditions for CSC separation.

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Fig. 1.
Chemical structures of 4-methylumbelliferyl sugar derivatives separated in this study.

Fig. 2.
Schematic illustration of the satellite and the planetary motions in the present CSC instrument.

A. Left-handed multilayer coil

B. Conventional multilayer coil

Fig. 3.
Two different types of multilayer coils for CCC separation using the coil satellite centrifuge.

Fig. 4.

Separation of 4-methylumbelliferyl sugar derivatives obtained using the CSC with the conventional multilayer coiled columns in the satellite motion. Experimental conditions: apparatus: coil satellite centrifuge (CSC); column: conventional multilayer coil (1.5 mm I.D., 2.5 mm O.D., four layers, total capacity: 75.0 mL); solvent system: ethyl acetate/1-butanol/water; (A) (3 : 2 : 5, v/v), (B) (1 : 4 : 5, v/v); sample: 4-methylumbelliferyl sugar derivatives: (A) β-D-cellobioside (Cel) (1 mg) + β-D-glucopyranoside (Glc) (1 mg) + α-D-mannopyranoside (Man) (1 mg) + β-D-fucopyranoside (β-D-Fuc) (1 mg) + α-L-fucopyranoside (α-L-Fuc) (1 mg), (B) α-L-fuc (1 mg) + β-D-galactopyranoside (Gal) (1 mg) + Cel (1 mg); flow rate: (A) 0.8 mL/min, (B) 0.4 mL/min; fractionate: 2 min/tube; rotation speed (ω_1 , ω_2 , ω_3): (A) (300, 150, 150 rpm), (B) (300, 100, 200 rpm); rotating direction: (A) CCW (ω_1) – CCW (ω_2) – CCW (ω_3), (B) CW (ω_1) – CW (ω_2) – CW (ω_3). SF = solvent front.

$(\omega_1, \omega_2, \omega_3)$

(300, 300, 0 rpm)

(300, 0, 300 rpm)

A. Lower mobile phase (solvent system: EtOAc/1-BuOH/H₂O (3 : 2 : 5, v/v)) B. Upper mobile phase (solvent system : EtOAc/1-BuOH/H₂O (1 : 4 : 5, v/v)) **Fig. 5.**

Separation of 4-methylumbelliferyl sugar derivatives obtained using the CSC with the conventional multilayer coiled columns in the two different types of the planetary motion. Experimental conditions: apparatus: coil satellite centrifuge (CSC); column: common multilayer coil (1.5 mm I.D., 2.5 mm O.D., four layers, total capacity: 75.0 mL); solvent system: ethyl acetate/1-butanol/water; (A) (3 : 2 : 5, v/v), (B) (1 : 4 : 5, v/v); sample: 4-methylumbelliferyl sugar derivatives: (A) β -D-cellobioside (Cel) (1 mg) + β -D-glucopyranoside (Glc) (1 mg) + α -D-mannopyranoside (Man) (1 mg) + β -D-fucopyranoside (β -D-Fuc) (1 mg) + α -L-fucopyranoside (α -L-Fuc) (1 mg), (B) α -L-fuc (1 mg) + β -D-galactopyranoside (Gal) (1 mg) + Cel (1 mg); flow rate: (A) 0.8 mL/min, (B) 0.4 mL/min; fractionate: 2 min/tube; rotating direction: (A) CCW (ω_1) – CCW (ω_2) – CCW (ω_3), (B) CW (ω_1) – CW (ω_2) – CW (ω_3). SF = solvent front.

Fig. 6.
Effect of the rotation speeds, ω_2 and ω_3 , on the peak resolution and the stationary phase retention using the CSC with the conventional multilayer coiled columns.

Fig. 7.
Effect of the hydrophobicity of the *n*-hexane/ethyl acetate/1-butanol/methanol/water systems on the stationary phase retention under satellite and planetary motions.

Fig. 8.

Effect of the rotation speeds, ω_2 and ω_3 , on the stationary phase retention with the upper mobile phase of the *n*-hexane/ethyl acetate/1-butanol/methanol/water system at four different volume ratios.

Fig. 9. Schematic illustration of the acceleration sensor set on the column holder. The acceleration change in one minute was measured after the stable rotation was achieved at the desired combination of rotation speeds.

Fig. 10.

The acceleration tracks obtained using the acceleration sensor with eight different combinations of rotation speeds at the CCW (ω_1) – CCW (ω_2) – CCW (ω_3) rotation direction for the lower mobile phase.

A. (300, 100, 200)

B. (300, 135, 165)

C. (300, 147, 153)

D. (300, 150, 150)

E. (300, 153, 147)

F. (300, 160, 140)

G. (300, 165, 135)

H. (300, 200, 100)

Fig. 11.

The acceleration tracks obtained using the acceleration sensor with eight different combinations of rotation speeds at the CW (ω_1) – CW (ω_2) – CW (ω_3) rotation direction for the upper mobile phase.

Table 1

The specific gravity and the settling time of the *n*-hexane/ethyl acetate/1-butanol/methanol/water system at the various volume ratio with the wide range of the hydrophobicity

No.	Solvent system					Specific gravity			Settling time ⁶⁾ (s)
	<i>n</i> -Hexane	Ethyl acetate	1-Butanol	Methanol	Water	Upper phase	Lower phase	Difference	
1	10	0	0	5	5	<0.700	0.926	> 0.226	5
2	9	1	0	5	5	<0.700	0.926	> 0.226	8
3	8	2	0	5	5	<0.700	0.926	> 0.226	14
4	7	3	0	5	5	0.707	0.931	0.224	20
5	6	4	0	5	5	0.727	0.931	0.204	22
6	5	5	0	5	5	0.747	0.927	0.180	26
7	4	5	0	4	5	0.767	0.937	0.170	28
8	3	5	0	3	5	0.794	0.950	0.156	30
9	2	5	0	2	5	0.825	0.966	0.141	30
10	1	5	0	1	5	0.857	0.982	0.125	30
11	0	5	0	0	5	0.908	1.004	0.096	32
12	0	4	1	0	5	0.898	0.998	0.100	20
13	0	3	2	0	5	0.889	0.997	0.108	15
14	0	2	3	0	5	0.878	0.994	0.116	12
15	0	1	4	0	5	0.865	0.992	0.127	14
16	0	0	5	0	5	0.825	0.991	0.166	17

The specific gravity was measured by the gravimeter at room temperature.