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Design of a novel coil satellite centrifuge and its performance on counter-current chromatographic separation of 4-methylumbelliferyl sugar derivatives with organic-aqueous two-phase solvent systems

Kazufusa Shinomiya^{a,*}, Koji Tokura^b, Emiru Kimura^a, Midori Takai^a, Naoki Harikai^a, Kazunori Yoshida^b, Kazuhiro Yanagidaira^b, and Yoichiro Ito^c

^aSchool of Pharmacy, Nihon University, 7-7-1, Narashinodai, Funabashi-shi, Chiba 274-8555, Japan

^bCollege of Science and Technology, Nihon University, 7-24-1, Narashinodai, Funabashi-shi, Chiba 274-8501, Japan

^cLaboratory of Bioseparation Technology, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, National Institutes of Health, 10 Center Drive, Building 10, Room 8N230, Bethesda, MD 20892-1762, USA

Abstract

A new high-speed counter-current chromatograph, named coil satellite centrifuge (CSC), was designed and fabricated in our laboratory. The CSC apparatus produces the satellite motion such that the coiled column simultaneously rotates around 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). In order to achieve this triplicate rotary motion without twisting of the flow tube, the rotation of each axis was determined by the following formula: $\omega_1 = \omega_2 + \omega_3$. This relation enabled to lay out the flow tube by two different ways, the SS type and the JS type. In the SS type, the flow tube was introduced from the upper side of the apparatus into the sun axis of the first rotary frame and connected to the planet axis of the second rotary frame like a double letter SS. In the JS type, the flow tube was introduced from the bottom of the apparatus into the sun axis reaching the upper side of the planet axis an inversed letter J, followed by distribution as in the SS type. The performance of the apparatus was examined on separation of 4-methylumbelliferyl (MU) sugar derivatives as test samples with organic-aqueous two-phase solvent systems composed of ethyl acetate/1-butanol/water (3 : 2 : 5, v/v) for lower phase mobile and (1 : 4 : 5, v/v) for upper phase mobile. With lower phase mobile, five 4-MU sugar derivatives including β -D-cellobioside (Cel), β -D-glucopyranoside, α -D-mannopyranoside, β -D-fucopyranoside and α -L-fucopyranoside (α -L-Fuc) were separated with the combined rotation around each axis at counterclockwise (CCW) (ω_1) – CCW (ω_2) – CCW (ω_3) by the JS type flow tube distribution. With upper phase mobile, three 4-MU sugar derivatives including α -L-Fuc, β -D-galactopyranoside and Cel were separated with the combined rotation around each axis at clockwise (CW) (ω_1) – CW (ω_2) – CW (ω_3) by the JS type flow tube distribution. A series of experiments on peak resolution and stationary phase retention revealed

*Corresponding author. Fax: +81-47-465-2158, shinomiya.kazufusa@nihon-u.ac.jp (K. Shinomiya).

that better partition efficiencies were obtained at the flow rate of 0.5 mL/min (column 1) and 0.8 mL/min (column 2) for lower phase mobile and 0.2 mL/min (column 1) and 0.4 mL/min (column 2) for upper phase mobile when using the left-handed multilayer coil (total capacity: 57.0 mL for column 1 and 75.0 mL for column 2) under the rotation speeds of approximately $\omega_1 = 300$ rpm, $\omega_2 = 150$ rpm and $\omega_3 = 150$ rpm.

Keywords

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

1. Introduction

Counter-current chromatography (CCC) is one of liquid-liquid partition chromatography, which eliminates the use of solid support matrix. This advantage gives the recovery of all samples charged into the CCC system without loss or denaturation. In order to improve the partition efficiency, many CCC instruments have been developed in the past [1–3]. Among them, high-speed CCC has proven most effective for the separation of various compounds [4]. Fig. 1 illustrates the schematic drawing of the two different types of high-speed CCC. They employ the planetary motion of coiled separation column to achieve good resolution of compounds, where the rotation pattern is different from each other. In the type-J coil planet centrifuge (CPC), the coiled column rotates about its own axis while simultaneously revolving around the central axis. This simple planetary motion is effective for the separation of various chemical compounds when using organic-aqueous two-phase solvent systems. Recently, the universal high-speed CCC, which can perform the type-J and type-I planetary motions, has been developed in our laboratory [5]. However, these high-speed CCC systems failed to retain polar solvent system such as aqueous-aqueous polymerphase system which is useful for separation proteins. In order to improve performance of the type-J coil planet centrifuge, the configuration of the conventional multilayer coil was modified to a spiral to utilize the radial centrifugal force for retention of the stationary phase [6–8]. The satisfactory retention of the polar solvent systems can also be effected by non-synchronous coil planet centrifuges while its highly complex mechanical desing detars the development of the commercial model [9,10] In addition the cross-axis CPC can retain polar two-phase solvent systems. It provides the complicated planetary motion, such that the column holder rotates about its horizontal axis while revolving around the vertical central axis of the apparatus at the same angular velocity [11, 12]. This unique motion is effective for the separation of high molecular weight compounds such as proteins and enzymes using aqueous-aqueous polymer phase systems. In our laboratory, both floor and bench-top models of the type X-1.5L cross-axis CPC have been fabricated and successfully applied to the separation of proteins using aqueous-aqueous polymer phase systems [13, 14].

In order to improve the partition efficiency of type-J high-speed CCC for separation of polar compounds, a series of chromatographic parameters including the increase of interface area by mixing of two phases for effective partitioning, the quick formation of two layers under the centrifugal force field and the suitable flow rate of the mobile phase should be established in the rotating coil. The present paper describes the design and the fabrication of

a new coil planet centrifuge named the coil satellite centrifuge (CSC), which produces the satellite motion such that the coiled separation column simultaneously rotates around the sun axis (ω_1), the planet axis (ω_2) and the satellite axis (ω_3) where $\omega_1 = \omega_2 + \omega_3$ to prevent the flow tube from twisting. When $\omega_2 = 0$ ($\omega_1 = \omega_3$), the system becomes identical to the high-speed CCC which can produce an efficient separation with a conventional organic-aqueous two-phase solvent systems, but fails to retain polymer phase systems which are useful for separation of proteins. On the other hand, when $\omega_3 = 0$ ($\omega_1 = \omega_2$), it becomes identical to the horizontal flowthrough CPC which can separate proteins at a low flow rate [15]. The present system is a hybrid between these two systems which may improve the performance for the separation of polar compounds by adjusting the rotation ratio between the planet (ω_2) and the satellite (ω_3). The partition efficiency of the apparatus was examined using the separation of 4-methylumbelliferyl sugar derivative mixtures with a polar organic-aqueous two-phase solvent system composed of ethyl acetate/1-butanol/water previously reported [5].

2. Apparatus

The CSC instrument employed in the present studies was newly constructed at the Machining Technology Center of Nihon University, Chiba, Japan. Fig. 2 illustrates the photograph of the CSC instrument. Two second rotary frames on the first rotary frame revolve around the sun axis while rotating about the planet axis, and four satellite columns set on the second rotary frame revolve around the planet axis and simultaneously rotate about their own axis. The design and fabrication of the apparatus are described below.

2.1. Satellite motion of coiled column

Fig. 3 shows the satellite motion of four coiled columns for CSC with single tube distribution without twisting. The CSC instrument has three different rotation axes including sun axis, planet axis and satellite axis. The satellite axis corresponds to the column rotation axis. Each angular velocity is expressed as ω_1 for the sun axis, ω_2 and ω_4 for the planet axis, and ω_3 , ω_5 , ω_6 and ω_7 for the satellite axis. When the sun axis revolves counterclockwise (CCW) viewed from the top of the apparatus, the planet axes 1 and 2 rotate CCW and clockwise (CW) respectively, and then the satellite axes 1 and 3 rotate CCW and the satellite axes 2 and 4 rotate CW, i.e., the neighboring columns rotate in the opposite direction to each other. In order to avoid twisting of the flow tube, the rotation of each axis is determined by the following formula: $\omega_1 + \omega_2 + \omega_3 = 0$ and $\omega_3 + \omega_7 = 0$, $-\omega_1 + \omega_4 + \omega_5 = 0$ and $\omega_5 + \omega_6 = 0$, and $\omega_6 + \omega_7 = 0$, as indicated in the figure.

2.2 Design of the CSC instrument

Fig. 4 illustrates the original design of the CSC instrument. Each axis rotates according to the equation: $\omega_1 + \omega_2 + \omega_3 = 0$. When the sun axis of the central shaft revolves CCW at ω_1 by the main motor and the satellite axis of the column rotates CW at ω_A by the side motor, the planet axis rotates CW at ω_2 as calculated from $-\omega_1 + \omega_A$, and then ω_3 corresponds to $-\omega_A$.

In this case, the first rotary frame revolves CCW, then the second rotary frame set on the first rotary frame and the column set on the second rotary frame should rotate CW in order to avoid the twisting of the flow tube. When the side motor rotates at ω_A during the main motor drives the first rotary frame at ω_1 , gear A connected with the side motor rotates ω_A in the opposite direction and gear B set on the first rotary frame rotates at $\omega_1 - \omega_A$. Then gear C engaged to gear B rotates the planet axis of the second rotary frame at $-(\omega_1 - \omega_A) = -\omega_1 + \omega_A$, and the column rotates at $\omega_3 = -\omega_A$. As the result, the total angular velocities of the CSC instrument are calculated as follows: $\omega_1 + \omega_2 + \omega_3 = \omega_1 + (-\omega_1 + \omega_A) + (-\omega_A) = 0$.

2.3 Flow tube distribution without twisting

Fig. 5 illustrates the cross-sectional view through the central axis of the CSC instrument. In order to avoid twisting, two different types of flow tube distribution can be used each for the specific combination of the rotation rates between three axes. In the SS type shown in Fig. 5A, the flow tube introduced from the upper side of the apparatus into the sun axis of the first rotary frame exits the side hole and passes through the planet axis and the satellite axis, forming a double letter SS. In the JS type shown in Fig. 5B, the flow tube is introduced from the bottom of the apparatus into the sun axis reaching the upper side of the planet axis like an inversed letter J, and then follows the distribution as in the SS type.

3. Experimental

3.1 Preparation of coiled column assembly

In order to evaluate the partition efficiency on the newly fabricated CSC instrument, the multilayer coil assembly was used, because it has been widely used for the type-J CPC in high-speed CCC. Two different total column volumes were used in the present studies, 57.0 mL for column 1 and 75.0 mL for column 2. Each multilayer coil was prepared by tightly winding a piece of 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 three (for column 1) or four (for column 2) tight left-handed 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. After each layer was completed, the layer was wrapped with an adhesive tape and the tubing was straightly returned to the top side from the bottom side to resume winding the next layer in the same direction. It results in a multilayer coil assembly composed of entirely left-handed coils, which is different from that commonly used in the type-J CPC. Four left-handed coil assemblies were connected in series with flow tubes, where two coil assemblies were diagonally located on each second rotary frame that is symmetrically set around the planet axis.

3.2 Reagents

All organic solvents and reagents used for CSC separation were of reagent grade. 4-Methylumbelliferyl sugar derivatives used as test samples were purchased 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 Wako Pure Chemicals (Osaka, Japan).

3.3 Preparation of two-phase solvent system and sample solutions

The organic-aqueous two-phase solvent system used in the present studies was composed of ethyl acetate/1-butanol/water (3 : 2 : 5, v/v) for lower phase mobile and (1 : 4 : 5, v/v) for upper phase mobile [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 CSC separation.

3.4 CSC separation of 4-methylumbelliferyl sugar derivatives

Each separation was initiated by completely filling the column with the stationary phase, followed by injecting the sample solution (ca. 1 mL) into the column inlet with a syringe. Then, 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) while the column was rotated with the desired combination of the rotation speeds of ω_1 , ω_2 and ω_3 . The rotation direction of each axis is CCW (sun axis) – CCW (planet axis) – CCW (satellite axis) for lower phase mobile and CW (sun axis) – CW (planet axis) – CW (satellite axis) under the JS type flow tube distribution. The effluent from the outlet of the column was collected into test tubes at 2 minutes/tube using a fraction collector (Model CHF 100AA, Advantec, Tokyo, Japan).

3.5 Analysis of CSC fractions

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

3.6 Evaluation of partition efficiency

The efficiencies in sugar derivative separations were evaluated by peak resolution (R_s) computed from the chromatogram and stationary phase retention measured after each separation.

4. Results and discussion

4.1 CSC separation of 4-methylumbelliferyl sugar derivatives

Partition efficiency of the CSC instrument was examined using the separation of 4-methylumbelliferyl sugar derivatives. The CSC separation was performed by the two different total column volumes of left-handed multilayer coil assemblies, 57.0 mL for column 1 and 75.0 mL for column 2. The organic-aqueous two-phase solvent system used in the present studies was composed of ethyl acetate/1-butanol/water (3 : 2 : 5, v/v) for the lower phase mobile and (1 : 4 : 5, v/v) for the upper phase mobile as reported earlier [5]. As described above, the CSC instrument employs two different flow tube distributions, the SS

type and the JS type. Among these, the JS type was selected to perform the CSC separation where the identical rotation direction can be set up between three axes.

Fig. 6 illustrates the CSC separation of 4-methylumbelliferyl sugar derivatives obtained using the JS type CSC at the rotation speeds, $\omega_1 = 300$ rpm, $\omega_2 = 150$ rpm and $\omega_3 = 150$ rpm. With lower phase mobile shown in Fig. 6A, five sugar derivatives were separated with the combined rotation at CCW (ω_1) – CCW (ω_2) – CCW (ω_3). In the upper chromatogram, column 1 (57.0 mL capacity) produced insufficient separation between two sugar derivatives, Man and β -D-Fuc, at the flow rate of 0.5 mL/min of the mobile phase. Using the higher volume of column 2 (75.0 mL capacity) improved separation of five sugar derivatives, especially the separation between Man and β -D-Fuc, at the flow rate of 0.8 mL/min of the mobile phase in the lower chromatogram. With upper phase mobile shown in Fig. 6B, three sugar derivatives were separated with the combined rotation at CW (ω_1) – CW (ω_2) – CW (ω_3). Satisfactory separation of these sugar derivatives were obtained in both columns 1 and 2 at the flow rate of 0.2 mL/min for column 1 and 0.4 mL/min for column 2, while column2 showed less retention of the stationary phase than column 1.

Table 1 summarizes the effect of flow rate of the mobile phase on peak resolution and stationary phase retention obtained using the JS type CSC with rotation speeds at $\omega_1 = 300$ rpm, $\omega_2 = 150$ rpm and $\omega_3 = 150$ rpm. With lower phase mobile, the decreased flow rate improved the peak resolution between each sugar derivative, while the stationary phase retention was almost constant at near 40 %. Improved separation of sugar derivatives were obtained at the higher volume of column 2. With upper phase mobile, the flow rate of 0.6 mL/min for column 1 and 0.8 mL/min for column 2 did not retain an enough amount of the stationary phase in the column, but the decreased flow rate produced the separation of sugar derivatives apparently due to the improved stationary phase retention. Higher retention of the stationary phase was obtained by the lower phase mobile than by the upper phase mobile. The stationary phase retention may depend on the difference in viscosity between the two phases where while the lower phase mobile yields higher retention than the upper phase mobile in hydrophobic organic-aqueous two-phase solvent systems.

4.2 Effect of rotation speeds of the planet axis and the satellite axis

Table 2 summarizes the effect of rotation speeds, ω_2 and ω_3 , on peak resolution and stationary phase retention obtained using the JS type CSC with the revolution speed at $\omega_1 = 300$ rpm. The separation of sugar derivatives was performed by the flow rate of 0.5 mL/min (column 1) and 0.8 mL/min (column 2) for lower phase mobile and 0.2 mL/min (column 1) and 0.4 mL/min (column 2) for upper phase mobile in order to achieve better peak resolutions within relatively shorter separation times. In both lower and upper phases used as the mobile phase, the satisfactory peak resolutions of sugar derivatives were obtained in rotation speeds of ω_2 and ω_3 at around 150 rpm. With lower phase mobile of column 1, slightly higher rotation speed of ω_2 than ω_3 yielded better peak resolution while in column 2 almost similar peak resolution was obtained between five sugar derivatives except that the rotation speeds were set at $\omega_2 = 100$ rpm and $\omega_3 = 200$ rpm. With upper phase mobile, the combined rotation at $\omega_2 = 153$ rpm and $\omega_3 = 147$ rpm enabled to achieve peak resolution and stationary phase retention in both columns, but slight changes of the rotation speeds to $\omega_2 =$

147 rpm and $\omega_3 = 153$ rpm failed to retain the lower stationary phase in both columns where all solutes were eluted at the solvent front without separation. The overall results suggest that satisfactory separation of 4-methylumbelliferyl sugar derivatives was performed using the combined rotation at $\omega_1 = 300$ rpm, $\omega_2 = 150$ rpm and $\omega_3 = 150$ rpm with the head to tail elution mode.

5. Conclusion

A new high-speed counter-current chromatograph, CSC, was designed and fabricated in our laboratory. The CSC apparatus produces the satellite motion such that the coiled column simultaneously rotates around the sun axis (ω_1), the planet axis (ω_2) and the satellite axis (the central axis of the column) (ω_3). In both lower and upper phases used as the mobile phase, better peak resolution of 4-methylumbelliferyl sugar derivatives was achieved using the organic-aqueous two-phase solvent systems composed of ethyl acetate, 1-butanol and water at lower rotation speeds of $\omega_1 = 300$ rpm, $\omega_2 = 150$ rpm and $\omega_3 = 150$ rpm. The overall results suggest that the CSC instrument proposed in the present study is useful for separation of various compounds at lower speeds of satellite motion.

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References

1. Mandava, NB.; Ito, Y. Countercurrent Chromatography: Theory and Practice. Marcel Dekker; New York: 1988.
2. Conway, WD. Countercurrent Chromatography: Apparatus, Theory & Applications. VCH; Weinheim: 1990.
3. Menet, J-M.; Thiebaut, D. Countercurrent Chromatography. Marcel Dekker; New York: 1999.
4. Ito, Y.; Conway, WD. High-Speed Countercurrent Chromatography. Wiley-Interscience; Amsterdam: 1996.
5. Shinomiya K, Sato K, Yoshida K, Tokura K, Maruyama H, Yanagidaira K, Ito Y. Partition efficiencies of newly fabricated universal high-speed counter-current chromatograph for separation of two different types of sugar derivatives with organic-aqueous two-phase solvent systems. *J Chromatogr A*. 2013; 1322:74–80. [PubMed: 24267319]
6. Ito Y, Yang FQ, Fitze PE, Sullivan JV. Spiral disk assembly for high-speed countercurrent chromatography. *J Liq Chrom & Rel Technol* (Special Issue for CCC2002). 26(9&10):1355–1372. Ito, Y.; Yang, F.-Q.; P. Fitze; J. Powell; D. Ide: Improved spiral disk assembly for high-speed countercurrent chromatography. **J. Chromatogr. A** 1017 (2003) 71–81.
7. Ito Y, Qi L, Powell J, Sharpnack F, Metger H, Yost J, Cao X-L, Dong Y-M, Huo L-S, Zhu X, Li T. Mixer-settler countercurrent chromatography with a barricaded spiral disk assembly with glass beads. *J Chromatogr A* in CCC2006 Special Issue. 2007; 1151:108–114.
8. Ito Y. Spiral column design for separation of proteins by high-speed countercurrent chromatography. *Chem Eng Proc*. 2010; 49(7):782–792.
9. Ito Y, Bramblett GT, Bhatnagar R, Huberman M, Leive L, Cullinane LM, Groves W. Improved non-synchronous flow-through coil planet centrifuge without rotating seals. Principle and application. *Separation Science and Technology*. 1983; 18(1):33–48.
10. Ignatova SN, Hawes D, van den Heuvel R, Hewitson P, Sutherland IA. A new non-synchronous preparative counter-current centrifuge – the next generation of dynamic extraction/

chromatography devices with independent mixing and settling control, which offer a step change in efficiency. *J Chromatogr A*. 2010; 1217:34–39. [PubMed: 19945712]

11. Ito Y. Cross-axis synchronous flow-through coil planet centrifuge free of rotary seals for preparative countercurrent chromatography. Part I. Apparatus and analysis of acceleration. *Sep Sci Technol*. 1987; 22:1971–1987.
12. Ito Y. Cross-axis synchronous flow-through coil planet centrifuge free of rotary seals for preparative countercurrent chromatography. Part II. Studies on phase distribution and partition efficiency in coaxial coils. *Sep Sci Technol*. 1987; 22:1989–2009.
13. Shinomiya K, Muto M, Kabasawa Y, Fales HM, Ito Y. Protein separation by improved cross-axis coil planet centrifuge with eccentric coil assemblies. *J Liq Chromatogr Relat Technol*. 1996; 19:415–425.
14. Shinomiya K, Yanagidaira K, Ito Y. New small-scale cross-axis coil planet centrifuge. The design of the apparatus and its application to counter-current chromatographic separation of proteins with aqueous-aqueous polymer phase systems. *J Chromatogr A*. 2006; 1104:245–255. [PubMed: 16405899]
15. Ito Y, Oka H. Horizontal flow-through coil planet centrifuge equipped with a set of multilayer coils around the column holder. *J Chromatogr*. 1988; 457:393–397. [PubMed: 2854141]

Fig. 1.
Planetary motions of the type-J coil planet centrifuge (CPC) and the cross-axis CPC.

Fig. 2.
Photograph of coil satellite centrifuge (CSC).

Fig. 3.
Satellite motion of four coiled columns in CSC with single flow tube distribution.

Fig. 4.
Design of the instrument of the coil satellite centrifuge (CSC).

Fig. 5.
Two different types of flow tube distribution without twisting for the CSC.

Fig. 6.

Separation of 4-methylumbelliferyl sugar derivatives obtained using the JS type CSC at the rotation speeds, $\omega_1 = 300$ rpm, $\omega_2 = 150$ rpm and $\omega_3 = 150$ rpm.

Experimental conditions: apparatus: coil satellite centrifuge (CSC); column: multilayer coil (left-handed, 1.5 mm I.D., 2.5 mm O.D., 3 layers for column 1, 4 layers for column 2, total capacity: 57.0 mL for column 1, 75.0 mL for column 2); flow tube distribution: JS type; 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) 0.5 mL/min for column 1 and 0.8 mL/min for column 2, (B) 0.2 mL/min for column 1 and 0.4 mL/min for column 2; fractionate: 2 min/tube; rotation speed: $\omega_1 = 300$ rpm, $\omega_2 = 150$ rpm and $\omega_3 = 150$ 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.

Table 1

Effect of the flow rate of mobile phase on the peak resolution and the stationary phase retention obtained using the JS type CSC with the rotation speeds at $\omega_1 = 300$ rpm, $\omega_2 = 150$ rpm and $\omega_3 = 150$ rpm

A. Lower phase mobile					
Flow rate (mL/min)	Peak resolution (Rs)			Stationary phase retention (%)	
	Cel/Glc	Glc/Man	Man/ β -D-Fuc	β -D-Fuc/ α -L-Fuc	
Column 1 (volume: 57.0 mL)					
0.6	2.6	1.2	0.5	1.3	40.4
0.5	2.6	1.2	0.7	1.1	36.8
0.4	2.9	1.5	0.8	1.1	42.9
Column 2 (volume: 75.0 mL)					
1.0	2.8	1.6	0.9	1.2	36.3
0.8	3.1	1.5	1.0	1.3	39.7
0.6	3.8	1.8	1.2	1.5	42.3

B. Upper phase mobile					
Flow rate (mL/min)	Peak resolution (Rs)			Stationary phase retention (%)	
	α -L-Fuc/Gal	Gal/Cel			
Column 1 (volume: 57.0 mL)					
0.6	— ^{*I}	— ^{*I}	— ^{*I}	— ^{*I}	— ^{*I}
0.4	0.6	0.8		13.3	
0.2	1.4	1.4		18.3	
Column 2 (volume: 75.0 mL)					
0.8	— ^{*I}	— ^{*I}	— ^{*I}	— ^{*I}	— ^{*I}
0.6	0.7	0.9		8.0	
0.4	1.4	1.8		15.3	

^{*I} All solutes were eluted in the solvent front without retaining in the column.

Effect of the rotation speeds, ω_2 and ω_3 , on the peak resolution and the stationary phase retention obtained using the JS type CSC with the revolution speed at $\omega_1 = 300$ rpm

Table 2

A. Lower phase mobile									
Rotation speed (rpm)		Peak resolution (Rs)							
ω_2	ω_3	Cel/Glc		Glc/Man		Man/ β -D-Fuc		β -D-Fuc/ α -L-Fuc	
		Column 1	Column 2	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
100	200	2.8	—*1	1.1	—*1	0.5	—*1	1.0	—*1
200	100	2.3	3.0	1.0	1.6	0.5	1.0	1.0	1.2
147	153	2.8	3.4	1.3	1.8	0.7	1.0	1.1	1.2
153	147	2.7	3.1	1.2	1.6	0.6	1.0	1.1	1.2
135	165	2.6	3.5	1.2	1.7	0.6	1.0	1.1	1.3
165	135	2.9	3.4	1.4	1.5	0.7	1.0	1.1	1.2
B. Upper phase mobile									
Rotation speed (rpm)		Peak resolution (Rs)							
ω_2	ω_3	α -L-Fuc/Gal		Gal/Cel		Stationary phase retention (%)			
		Column 1	Column 2	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
147	153	—*2	—*2	—*2	—*2	—*2	—*2	—*2	—*2
153	147	1.2	1.0	1.5	1.3	23.6	13.0		

*1 The rotation speeds were not mechanically set due to the stress to the apparatus.

*2 All solutes were eluted in the solvent front without retaining in the column.