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Effect of Coriolis force on counter-current chromatographic separation by centrifugal partition chromatography

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Abstract

The effect of Coriolis force on the counter-current chromatographic separation was studied using centrifugal partition chromatography (CPC) with four different two-phase solvent systems including *n*-hexane-acetonitrile (ACN); *tert*-butyl methyl ether (MtBE)–aqueous 0.1% trifluoroacetic acid (TFA) (1:1); MtBE-ACN–aqueous 0.1% TFA (2:2:3); and 12.5% (w/w) polyethylene glycol (PEG) 1000–12.5% (w/w) dibasic potassium phosphate. Each separation was performed by eluting either the upper phase in the ascending mode or the lower phase in the descending mode, each in clockwise (CW) and counterclockwise column rotation. Better partition efficiencies were attained by the CW rotation in both mobile phases in all the two-phase solvent systems examined. The mathematical analysis also revealed the Coriolis force works favorably under the CW column rotation for both mobile phases. The overall results demonstrated that the Coriolis force produces substantial effects on CPC separation in both organic–aqueous and aqueous–aqueous two-phase systems.
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1. Introduction

Centrifugal partition chromatography (CPC) was introduced by Murayama et al. [1] as one of the preparative counter-current chromatographic systems. This apparatus is generally classified into the hydrostatic equilibrium system as is the toroidal coil counter-current chromatography (CCC). In these hydrostatic CCC systems, the solute partitioning is carried out under a strong centrifugal force field where the mixing of the two phases is entirely relied on the flow pattern of the mobile phase in the separation column. Consequently, the partition efficiency of the system is quite sensitive to the physical parameters which alter the flow pattern of the mobile phase.

Recently, Ito and co-workers reported the effect of the Coriolis force on the partition efficiency in the toroidal coil CCC centrifuge where the sharpness of the elution peak varied according to the direction of the coil rotation [2–4].

However, this effect was observed only in the protein separation with a viscous aqueous–aqueous polymer phase system, while no measurable effect was detected in the separation of dipeptides with conventional organic–aqueous two-phase solvent systems. Marchal et al. also studied the influence of flow patterns using CPC equipped with a camera and a stroboscope to observe the flow of mobile phase in the rotating channel and emphasized the importance of Coriolis force on flow shape [5,6]. The present paper describes the effect of the Coriolis force on the partition efficiency of CPC separations using four different two-phase solvent systems together with a mathematical analysis of the Coriolis force.

2. Experimental

2.1. Apparatus

The CPC apparatus used in the present studies was obtained from Senshu (Tokyo, Japan) as a commercial unit originally fabricated by Sanki (Kyoto, Japan). The rotor consists of 12 layers of two disk units (total 24 disks) connected

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in series to make a total capacity of 220 ml including 15% of the dead space in the ducts. The separation disk (polyphenylene sulfide resin) made by injection molding contains 89 partition compartment (15 mm × 2.8 mm × 2.1 mm with a 88 μl capacity) serially connected with ducts (15 mm × 1 mm × 1 mm) [7].

2.2. Reagents

Hippuric acid (HA), *p*-amino-HA, *p*-methyl-HA, benzoic acid, tartaric acid, succinic acid, fumaric acid, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased from Wako (Osaka, Japan). 2-Nitrophenylhydrazine hydrochloride (ONPH) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Polyethylene glycol (PEG) 1000 (M_r 1000), cytochrome *c* (horse heart), myoglobin (horse skeletal muscle) and lysozyme (chicken egg) were purchased from Sigma. All other chemicals were of reagent grade.

2.3. Preparation of two-phase solvent systems and sample solutions

We selected four different two-phase solvent systems with a broad range in polarity. They are in an increasing order of polarity: (I) *n*-hexane-acetonitrile (ACN); (II) *tert*-butyl methyl ether (MTBE)–aqueous 0.1% trifluoroacetic acid (TFA) (1:1) [8]; (III) MTBE–ACN–aqueous 0.1% TFA (2:2:3) [9]; and (IV) 12.5% (w/w) PEG 1000–12.5% (w/w) dibasic potassium phosphate.

A set of test samples was selected for each two-phase solvent system according to their polarity. Each solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated after two clear layers formed. Sample solutions were prepared by dissolving each sample mixture in aliquots consisting of equal volumes of each phase of the two-phase solvent system used for separation. Test samples used for each two-phase solvent system are given in Table 1.

2.4. Separation procedure

Each separation was initiated by completely filling all the disks with the stationary phase followed by injection of the sample solution into the column inlet. Then, the mobile phase was pumped into the disk using a reciprocating pump (Model LC-10ADVP, Shimadzu Seisakusho, Kyoto, Japan), while the rotor was rotated at a desirable rotation speed. The effluent from the outlet was collected into test tubes using a fraction collector (Model SF-160, Advantec, Tokyo, Japan). The direction of the rotation was defined from the overview of the apparatus.

2.5. Analysis of fractions

Each collected fraction was diluted with aliquots of desirable solvent and the absorbance was measured using a spectrophotometer (Model UV-1600, Shimadzu Seisakusho).

For the detection of polar carboxylic acid, a 100 μl of each fraction was separated and evaporated by a centrifugal evap-

Table 1
Experimental conditions for separation using centrifugal partition chromatography with four different kinds of two-phase solvent systems

Solvent system	Sample		Revolution speed (rpm)	Flow rate (ml/min)	Fractionate (ml per tube)	Detection (nm)
	LP mobile	UP mobile				
(I) *n*-Hexane-ACN (1:1)	2-Naphthalenesulfonic acid Anthrone Naphthalene Acenaphthene *n*-Dodecylbenzene Dissolve with UP 2 ml and LP 5 ml, and submit 1 ml each to CPC	50 mg 40 mg 100 mg 200 mg 3 ml	1200	4.0	4.0	254
(II) MTBE–aqueous 0.1% TFA (1:1)	*p*-Aminohippuric acid 3 mg Hippuric acid 15 mg *p*-Methyl hippuric acid 15 mg	Benzoic acid 10 mg *p*-Methyl hippuric acid 10 mg Hippuric acid 10 mg	700	2.0	2.0	254
(III) MTBE–ACN–aqueous 0.1% TFA (2:2:3)	Tartaric acid 5 mg Succinic acid 5 mg Fumaric acid 5 mg		800	2.0	2.0	530 (EDC-ONPH method)
(IV) 12.5% (w/w) PEG 1000–12.5% (w/w) K_2HPO_4	Cytochrome *c* 5 mg Myoglobin 10 mg Lysozyme 10 mg	Lysozyme 10 mg Myoglobin 10 mg	1500	1.5	3.0	280

Abbreviations: LP, lower phase; UP, upper phase; ACN, acetonitrile; MTBE, *tert*-butyl methyl ether; TFA, trifluoroacetic acid; PEG, polyethylene glycol.
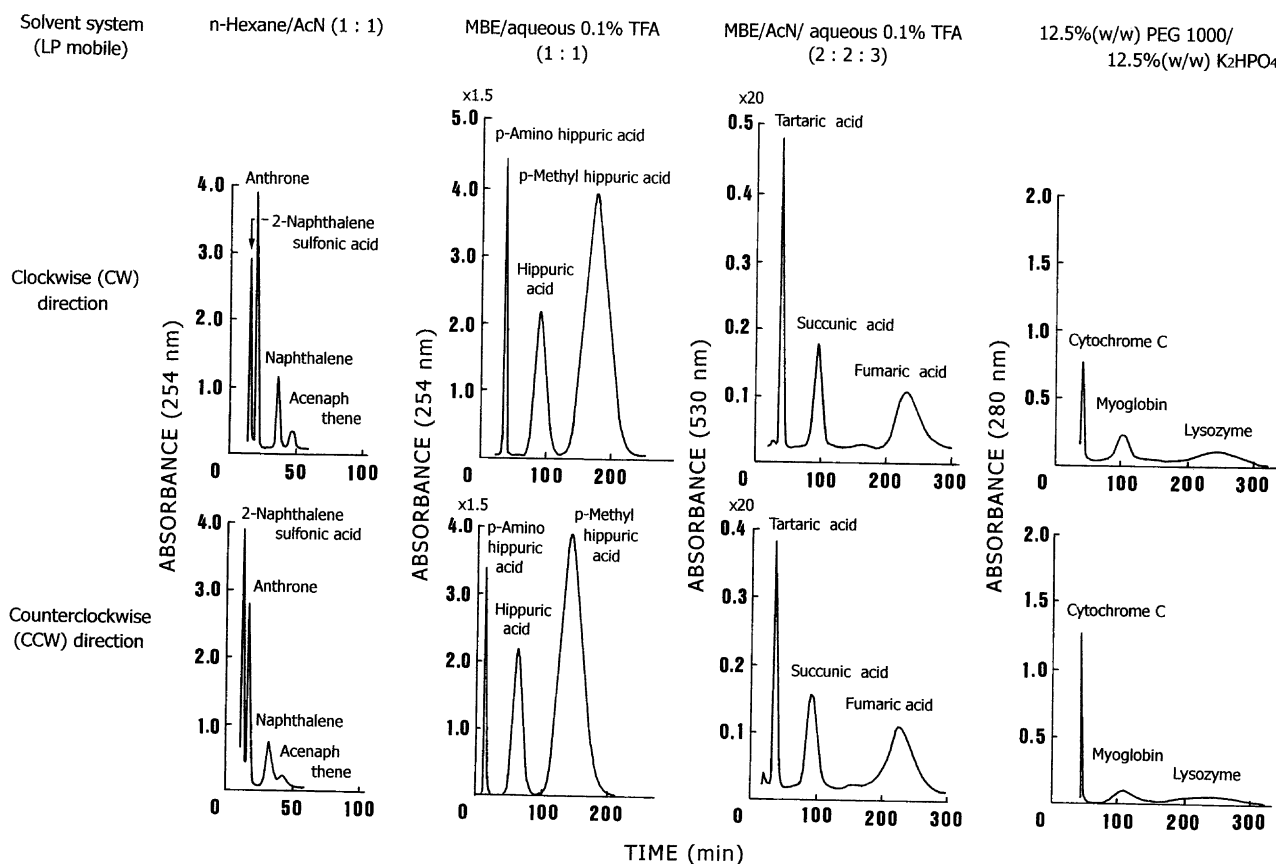
Fig. 1. Chromatograms obtained by CPC using four different two-phase solvent systems by eluting the lower phase in the descending mode. Experimental conditions are summarized in Table 1 of the text.

orator (Model CVE-3100, Tokyo Rikakikai, Tokyo, Japan), and then redissolved in 2 ml of water. This solution was submitted to the specific color reaction (EDC-ONPH method) as follows [10]: Two milliliters of the sample solution was

mixed with 1 ml each of 0.01 M ONPH in 0.4 M HCl and 0.15 M EDC in 4% (v/v) pyridine. The solution was incubated at 40 °C for 30 min, mixed with 1 ml of 1.5 M sodium hydroxide solution, and again incubated at 60 °C for 15 min

Table 2

Analytical values obtained by CPC using four different two-phase solvent systems with lower phase mobile of descending mode

Solvent system	Compound	Retention volume (CW/ CCW) (ml)	Partition coefficient, K^a (CW/CCW)	Resolution factor, R_s (CW/CCW)	Theoretical plate number, N (CW/CCW)	Retention of stationary phase (CW/CCW) (%)
(I) *n*-Hexane-ACN (1:1)	2-Naphthalenesulfonic acid	64/52	0.06/0.03	1.3/1.4	455.835	
	Anthrone	80/68	0.17/0.13	4.1/2.1	711/289	71.5/76.0
	Naphthalene	148/128	0.64/0.53	1.5/0.8	751/164	
	Acenaphthene	188/164	0.92/0.76		552/187	
(II) MTBE–aqueous 0.1% TFA (1:1)	*p*-Aminohippuric acid	74/24	0.19/0.06	2.7/3.0	698/74	
	Hippuric acid	180/124	0.87/0.60	1.5/1.7	115/78	78.0/94.0
	p-Methyl hippuric acid	350/284	1.96/1.45		80/70	
(III) MTBE–ACN–aqueous 0.1% TFA (2:2:3)	Tartaric acid	78/74	0.22/0.22	3.1/2.6	264/181	
	Succinic acid	190/184	0.94/0.90		201/136	72.0/79.0
	Fumaric acid	458/448	2.65/2.54		176/135	
(IV) 12.5% (w/w) PEG 1000–12.5% (w/w) K₂HPO₄	Cytochrome *c*	61.5/66	0.03/0.01	2.7/2.3	466/5378	
	Myoglobin	153/162	0.67/0.72	1.8/1.2	115/65	55.0/65.5
	Lysozyme	361.5/345	2.13/2.07		69/34	

[a] Partition coefficients were calculated according to the conventional formula: $K = (V_r - V_{sf})/(V_c - V_{sf})$, where V_r, V_c and V_{sf} indicate the retention volume of the solute peak, the column capacity and the retention volume of the solvent front, respectively. Abbreviations: CW, clockwise direction of rotation; CCW, counterclockwise direction.
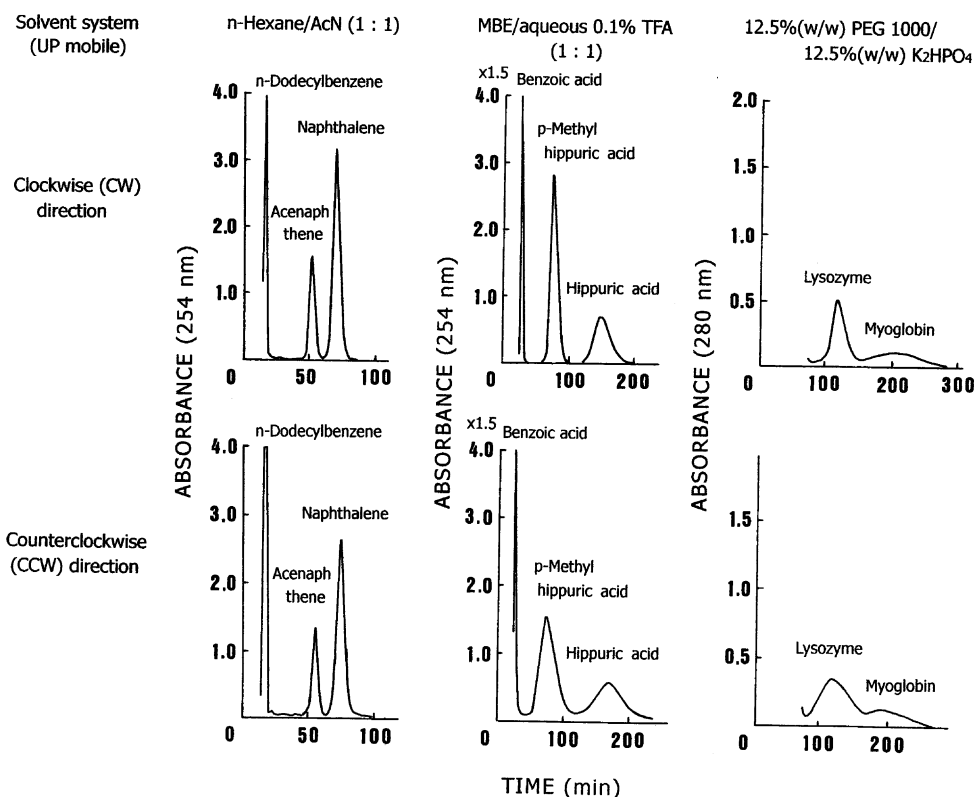
Fig. 2. Chromatograms obtained by CPC using three different two-phase solvent systems by eluting the upper phase in the ascending mode. Experimental conditions are summarized in Table 1 of the text.

to maximize color development. The absorbance was measured at 530 nm using a spectrophotometer.

3. Results and discussion

3.1. CPC Separations with four different two-phase solvent systems

Fig. 1 illustrates a set of CCC chromatograms obtained by CPC using four different two-phase solvent systems by eluting the lower phase in a descending mode. The two-phase solvent systems and experimental conditions applied in these separations are summarized in Table 1. For each solvent system the separations were performed by rotating the column in different directions of clockwise (CW; top row) and counter clockwise (CCW; bottom row) under otherwise identical conditions. The optimized conditions were established by measuring the maximum flow rate and rotation speed under the pressure limit of 60 kg/cm^2 because the increase of these two parameters yielded better theoretical plates as previously described by Marchal et al. [5,6].

Table 2 summarizes the analytical data computed from the chromatograms. Most of peak resolution and theoret-

Table 3

Analytical values obtained by CPC using three different two-phase solvent systems with upper phase mobile of ascending mode

Solvent system	Compound	Retention volume (CW/CCW) (ml)	Partition coefficient, K^a (CW/CCW)	Resolution factor, R_s (CW/CCW)	Theoretical plate number, N (CW/CCW)	Retention of stationary phase (CW/CCW) (%)
(I) *n*-Hexane–ACN (1:1)	*n*-Dodecylbenzene	68/64	0.11/0.08	5.0/5.2	185/164	
	Acenaphthene	208/220	1.05/1.14	1.7/1.7	534/484	69.0/74.0
	Naphthalene	280/296	1.54/1.65		508/589	
(II) MTBE–aqueous 0.1% TFA (1:1)	Benzoic acid	56/50	0.04/0.04	3.5/1.7	400/204	
	p-Methyl hippuric acid	152/148	0.68/0.67	2.0/1.4	186/34	72.0/74.0
	Hippuric acid	296/336	1.64/1.87		145/64	
(IV) 12.5% (w/w) PEG 1000–12.5% (w/w) K$_2$HPO$_4$	Lysozyme	172.5/174	0.71/0.73	0.9/0.5		45.0/40.0
	Myoglobin	303/294	2.08/1.99		31/16	

^{a} Partition coefficients were calculated according to the conventional formula: $K = (V_r - V_{sf})/(V_c - V_{sf})$, where V_r, V_c and V_{sf} indicate the retention volume of the solute peak, the column capacity and the retention volume of the solvent front, respectively. Abbreviations: CW, clockwise direction of rotation; CCW, counterclockwise direction.
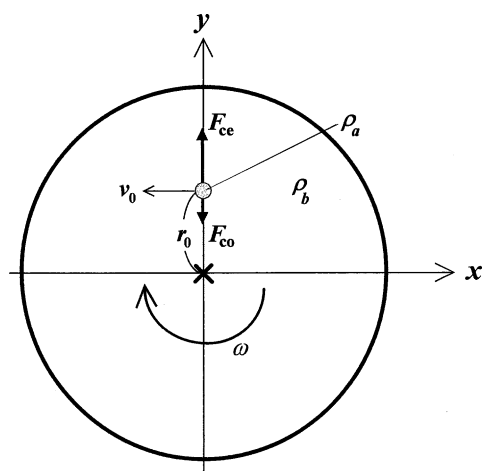
Fig. 3. Schematic drawing of physical forces acting in a compartment of the rotating disk.

ical plate numbers obtained by the CW column rotation are higher than those obtained by the CCW rotation in all solvent systems examined. It is interesting to note that the retention of the stationary phase exhibits the opposite trend

that the CCW rotation shows substantially higher value than the CW rotation for each group. This may indicate that the CCW rotation produces a more stable hydrodynamic condition resulting in less efficient mixing of the two phases in the column.

Fig. 2 similarly illustrates a set of CPC chromatograms obtained from three different two-phase solvent systems by eluting the upper phase in the ascending mode. As shown in Table 3, most of data obtained by the CW column rotation show higher efficiency than that obtained from the CCW column rotation. Here again higher retention of the stationary phase was obtained in the CCW rotation. The overall results strongly suggest that the Coriolis force affects the partition efficiency and retention of the stationary phase in all the two-phase solvent systems examined.

3.2. Mathematical analysis of Coriolis force in CPC separation

Fig. 3 illustrates the schematic drawing of physical forces acting in a compartment of the rotating disk. Using the lower phase as a mobile phase, the disk is filled with the upper
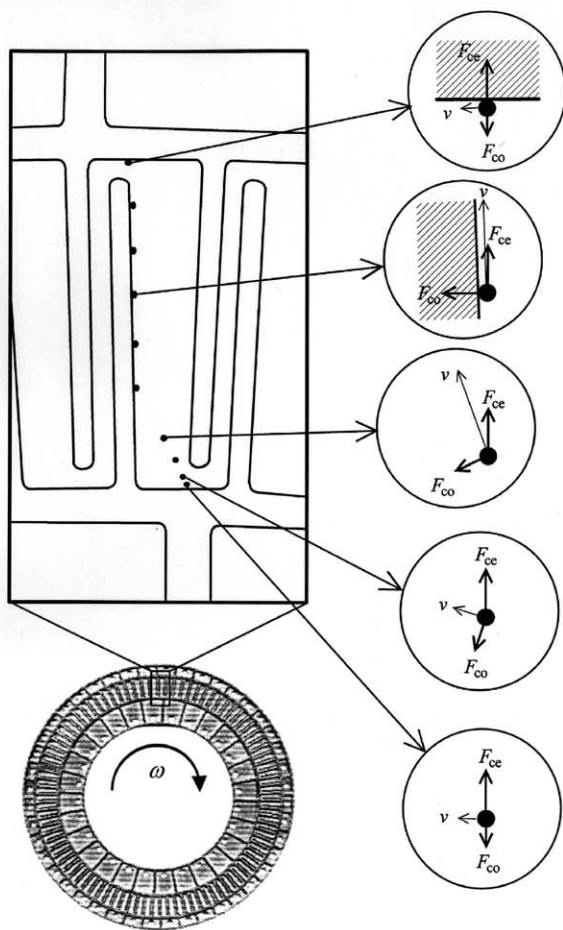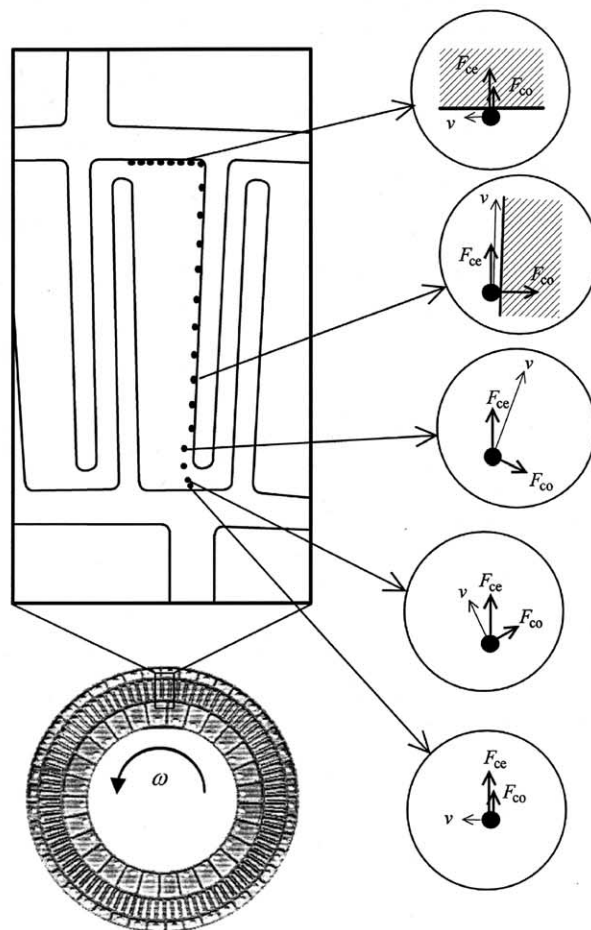

Fig. 4. Pathways of the droplets in the compartment by eluting the lower phase of the *n*-hexane-ACN system in the clockwise (A) and the counterclockwise (B) rotation.

Table 4
Measurements of densities and viscosities of four different two-phase solvent systems used in present studies

Solvent system	Phase	Density (g/ml)	Viscosity (mPa s)
(I) n-Hexane-ACN (1:1)	Upper	0.66	0.29
	Lower	0.76	0.33
(II) MTBE–aqueous 0.1% TFA (1:1)	Upper	0.74	0.35
	Lower	0.99	1.04
(III) MTBE–aqueous 0.1% TFA (2:2:3)	Upper	0.79	0.50
	Lower	0.96	1.10
(IV) 12.5% (w/w) PEG 1000–12.5% (w/w) K$_2$HPO$_4$	Upper	1.09	6.34
	Lower	1.16	1.81

stationary phase of density ρ_b and rotated at an angular velocity, ω, in the CW direction. The droplets of the mobile phase with density ρ_a flow through the compartment at a rate of v_0 from the initial position $(0, r_0)$. The centrifugal force, F_{ce}, and the Coriolis force, F_{co}, acting on the droplets may be expressed by the following equations:

$$F_{ce} = (\rho_a - \rho_b)Vr_0\omega^2 \tag{1}$$

and

$$F_{co} = 2\rho_a V\omega v_0 \tag{2}$$

where V is the volume of the droplet of the mobile phase. After a lapse of Δt (s), the droplet moves to point $P_1(v_0\Delta t, r_0)$ at a rate of:

$$v_{x1} = v_0 - 2\omega v_0\Delta t \tag{3}$$

and

$$v_{y1} = \left(1 - \frac{\rho_b}{\rho_a}\right)r_1\omega^2\Delta t \tag{4}$$

where $r_1 = (x_1^2 + y_1^2)^{1/2}$.

After another lapse of Δt (s), the droplet further moves to point P_2 $(x_1 + v_{x1}\Delta t, y_1 + v_{y1}\Delta t)$ at the following rate of:

$$v_{x2} = v_{x1} - 2\left(\frac{v_y}{|v_1|}\right)\omega v_1\Delta t \tag{5}$$

and

$$v_{y2} = v_{y1} + \left[\left(1 - \frac{\rho_b}{\rho_a}\right)r_2\omega - 2\left(\frac{v_x v_1}{|v_1|}\right)\right]\omega\Delta t \tag{6}$$

where $v_1 = (v_{x1}, v_{y1})$, $|v_1| = (v_{x1}^2 + v_{y1}^2)^{1/2}$, and $r_2 = (x_2^2 + y_2^2)^{1/2}$. The position of the droplet at each increment of Δt (s) may be determined by the above procedure.

Using n-hexane-ACN as a model system (see Table 4 for the physical properties of the two-phase solvent systems), the pathway of the lower phase droplets was computed in both CW (A) and CCW (B) column rotation by inserting the following values into the above equations: $\rho_a = 0.7629$ (g/ml); $\rho_b = 0.6566$ (g/ml); $r_0 = 7.5$ (cm); $v_0 = -6.667$ (cm/s), revolution speed $= 1200$ (rpm); and $\Delta_t = 0.0001$
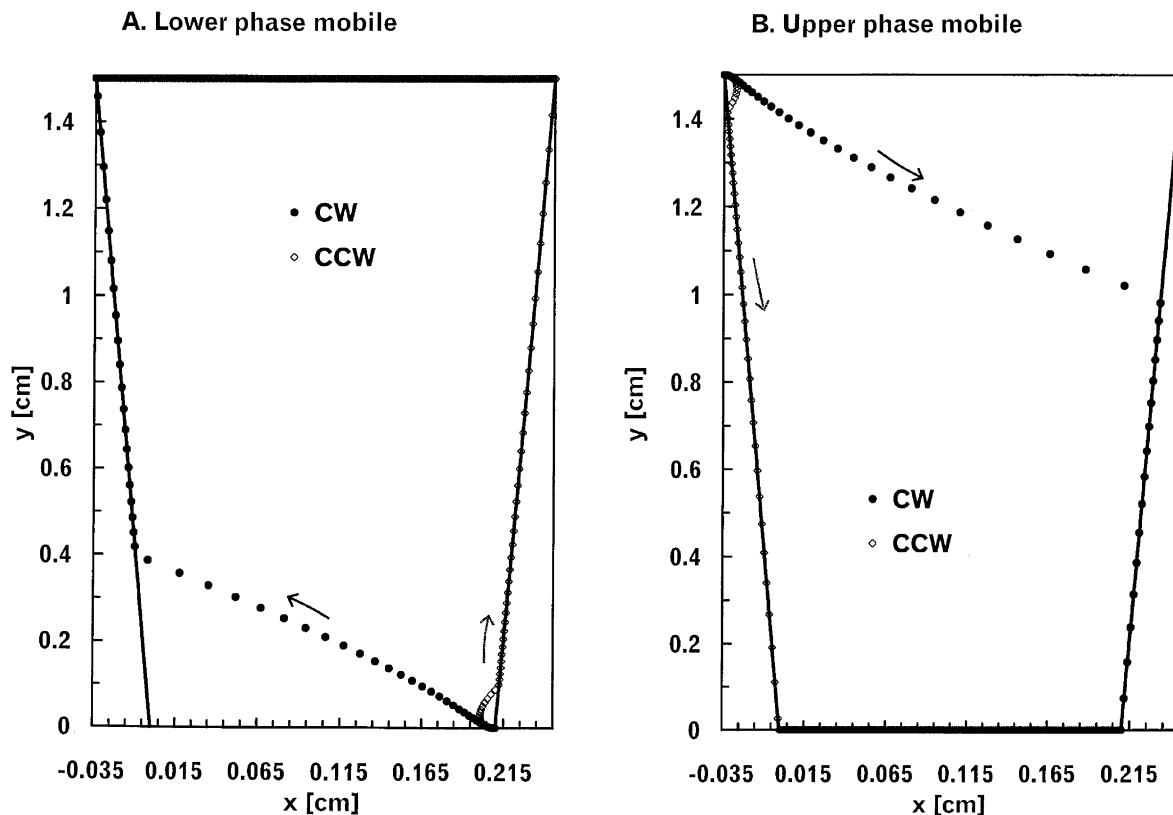

Fig. 5. Schematic drawing of the pathways of the droplets of the lower mobile phase (A) and the upper mobile phase (B).

(s). As shown in Fig. 4, the CW rotation forms multiple droplets of the lower mobile phase into the stationary phase providing a sufficient retention time for solute partitioning, while in the CCW rotation the mobile phase rapidly flows along the wall of the compartment. This assumption is supported by the observed retention time for each peak as described in Table 2.

Fig. 5 illustrates a schematic drawing of the pathways of the droplets of the lower mobile phase (A) and the upper mobile phase (B). Fig. 5 shows that the mobile phase droplets (black circle) are quickly reaching the wall across the chamber suggesting that the partition efficiency will be improved by adjusting the centrifugal force and/or flow rate so that the mobile phase droplets stay longer within the compartment before fitting the wall. These diagrams clearly show that the Coriolis force promotes the partition process in the CW direction, regardless of the choice of the mobile phase.

4. Conclusions

The effects of the Coriolis force on partition efficiency and stationary phase retention in CPC were investigated by changing the direction of column rotation. Overall results of the experiments revealed that the Coriolis force clearly affects the CPC separation in all solvent systems examined where the CW rotation yielded substantially higher partition efficiency and lower retention of the stationary phase regardless of the choice of the mobile phase. These findings are consistent with the mathematical analysis on the motion of the droplets in the partition compartment of the CPC apparatus. All the results presented in this work are valid with the machine used: the geometry of the channel and duct arrangement are critical. The best position for the two orifices of mobile phase entering and leaving the channels may well be in the middle top and rather than alternating left top and right bottom.

Acknowledgements

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