Retention of the Stationary Phase for High-Speed Countercurrent Chromatography

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High-speed countercurrent chromatography (HSCCC) is an emerging technique for preparative purification of a wide variety of solutes. Retention of the stationary phase is a crucial variable which is used for the derivation of the column efficiency, peak resolution, and solute retention. A mathematical model was proposed to describe the influences of operation conditions (flow rate, rotation speed), physical properties (density difference, viscosity, and interfacial tension), and instrument parameters (tube diameter, revolution radius) on the retention of the stationary phase, by building on the flow behavior of the two phases in the coiled column, laminar flow or droplet flow. The model parameters, together with the critical value at which the transition between the laminar flow and droplet flow occurs, were determined by the analysis of experimental data of the retention of the stationary phase measured in this work. Furthermore, the proposed model was used to predict the literature data of retention of the stationary phase for seven HSCCC apparatuses including preparative, semi-preparative, and analytical types, with the coiled column material of PTFE and stainless steel, and for 16 two-phase systems with 151 data points. The agreement between the predicted and the literature data is quite good with the total absolute deviation (AAD%) of 2.96% and the maximum deviation of 12.8%. © 2007 American Institute of Chemical Engineers *AIChE J,* 53: 1460–1471, 2007

Keywords: high-speed countercurrent chromatography (HSCCC), retention of stationary phase, laminar flow, droplet flow, flow rate, rotation speed

Introduction

High-speed countercurrent chromatography (HSCCC) is a versatile technology for analytical and preparative separations of chemical and pharmaceutical substances, especially for the preparative separation of natural products.[1–3] This chromatographic method is based on the difference in distribution of components over two immiscible liquid phases. There is no solid support and the liquid stationary phase is retained against the flow of the mobile phase due to the special action from multilayer helical coils of tubing constrained to rotate in the planetary motion. Clearly retention of stationary phase is essential if liquid–liquid chromatography is to take place. The retention of the stationary phase (S_f), expressed as the volume of stationary phase retained in the column over the total column volume (V_s/V_c), is one of the most important parameters in countercurrent chromatography (CCC), and it is used for the derivation of the column efficiency, peak resolution and solute retention.[4]

More than 20 different CCC apparatuses were designed by Ito.[5,6] From various possible designs, HSCCC is one of the arrangements, which best holds the stationary phase and allows the fastest and most effective separation. The scaling-

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up of HSCCC for industrial use is very promising and challenging. During the scaling-up of HSCCC for industrial preparation, the retention of the stationary phase should be the first variable to be considered. Therefore, it is very important to develop a mathematical model that will predict stationary phase retention as a function of the parameters that affect it.

The retention of the stationary phase (S_f) is dependent on three classes of parameters or variables: active parameters (that can be changed during an experiment): the mobile phase flow rate, rotation speed, and temperature; nonactive parameters (that can not be changed during an experiment): the geometrical parameters of the instrument, such as the revolution radius R, β-value (where β is the ratio of the coil radius r to the revolution radius R), tubing diameter, and tubing material; and the physical properties of the two-phase system: density and viscosity of each phase and interfacial tension between the two phases.

The influences of operation conditions and apparatus parameters on the retention of the stationary phase have been studied by many researchers.[7–11] A constant observation with different HSCCC apparatus is that S_f increases with the centrifuge rotation speed and decreases with the flow rate.[12] Du et al.[13] have shown that S_f decreases proportionally to the square root of the mobile phase flow rate: $S_f = A - B\sqrt{F}$. Wood et al.[14] have shown that this relationship can be explained by considering the coil planet centrifuge as a constant pressure pump, and using the Hagen–Poiseuille equation under the assumption that the mobile phase flow is laminar, as follows:

$$S_f = 1 - \frac{8}{d^2}\sqrt{\frac{2\mu_m L}{\pi \Delta P}}\sqrt{F} = \frac{6.383}{d^2}\sqrt{\frac{\mu_m L}{\Delta P}}\sqrt{F} \qquad (1)$$

where μ_m is the viscosity of the mobile phase; L is the length of the column and ΔP is the hydrodynamic pressure drop, d is the diameter of the column, and F is the flow rate of the mobile phase. However, the variable ΔP is unknown. Therefore, Eq. 1 could not be used to predict S_f at different operation conditions.

The influences of physical properties of two-phase solvent systems on the retention of the stationary phase have also been experimentally studied by a number of authors.[7–12,15,16] Parameters such as the polarity of solvent, density, and viscosity difference of the two phases, settling time, interfacial tension, and capillary wavelength have been considered. Berthod et al.[7,12,16] attempted to correlate S_f and physical properties of liquid systems. But no reliable correlations between S_f and physical properties were found.

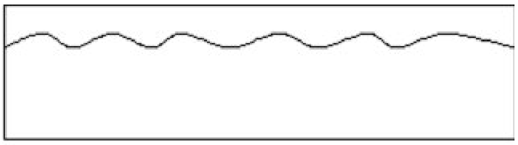
The objective of this article, therefore, is to investigate the relationship between the retention of the stationary phase and the influencing parameters, and to develop a mathematical model that could predict the retention of the stationary phase at different operation conditions with different two-phase solvent systems.

Model

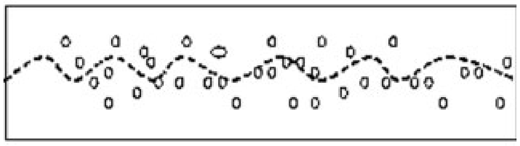
In HSCCC, the column holder rotates about its own axis and revolves around the centrifuge axis at the same angular velocity in the same direction. This type-J synchronous planetary motion generates a heterogeneous distribution of the centrifugal force vectors. Each turn of the coil bears a pair of key nodes where the Archimedean screw force reverses its direction with respect to the coil. The proximal key node triggers the movement of the lighter phase toward the head and the distal key node triggers the movement of the heavier phase toward the head. The forces acting at these two key nodes show different magnitude. Magnitude of the force acting at the distal key node on the coil always exceeds that acting at the proximal key node on the coil while both forces gain their strength as the helical diameter of the coil increases. This asymmetry of the force field produces the unilateral hydrodynamic distribution of the two-phase solvent systems.[17]

Because of the intriguing acceleration field, at the distal key node the two phases are separated as two layers. Next moment, the heavier phase moves toward the head and the lighter phase in the opposite direction (CC-I). Then, at the proximal key node the two phases mix together. Next moment, the lighter phase moves to the head and the heavier phase in the opposite direction, and undergo the second countercurrent (CC-II). The next moment the two phases are again separated at the distal key node. The above two countercurrent processes (CCs-I and II) are quite different not only in the direction of the flow but also in their flow pattern, i.e., in CC-I process the two-phase solvent system flows more like stratified film while in CC-II process it flows more like droplets. This fact bears an extremely important implication that hydrodynamic motion in the coil has two kinds of flow regime, the stratified film pattern defined as laminar flow, and the droplets pattern defined as droplet flow. Their schematic diagrams are shown in Figures 1a, b, respectively.

In a two-phase solvent system with short settling time, the droplet flow in CC-II tends to turn to laminar flow, and the laminar flow will be the principal flow regime. Conway had observed this kind of laminar flow directly by stroboscopic.[4] The stroboscopic observation revealed that the column was divided into two distinct zones, the mixing zone and the settling zone. The formation of a mixing zone (area of intense agitation) occurs in a portion of tubing in each helical turn closest to the central axis of revolution. The remainder of each helical turn represents a settling zone (formation of two layers) in which the lower phase occupies the peripheral half



（a） laminar flow



（b） droplet flow

Figure 1. Flow regimes of the two phases in the column of HSCCC.

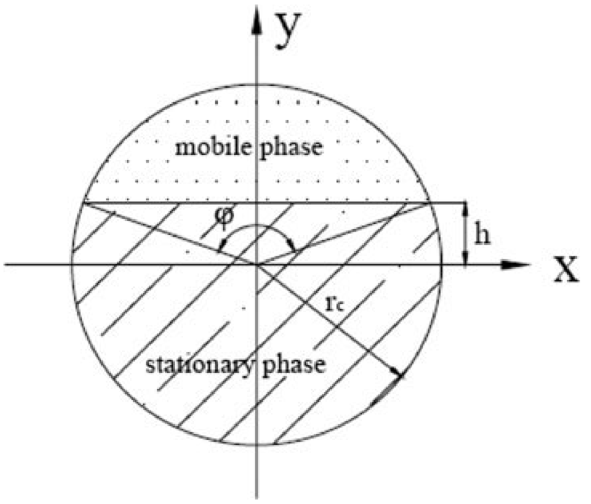


Figure 2. Stratified laminar flow in column of HSCCC.

of each helical turn while the upper phase occupies the innermost half.

On the other hand, in a system with long settling time, and the droplet flow becomes more important, at this time, the principal flow regime in the column can be considered as droplet flow. Fedotov et al.[18,19] considered that the stationary phase is retained in the column as separate elements having the shape of coarse elongated drops.

Therefore, the model of retention of the stationary phase is based on the above two flow behaviors.

Laminar flow

Assumptions:

(1) The interface between the mobile and stationary phases can be considered as a solid and moveable boundary in which the interface coincides with the horizontal diameter plane.

(2) The mixing zones observed by Conway[4] do not interfere significantly with the retention of the stationary phase.

(3) Helically or spirally wound tubing can be treated as straight tubing given that the bend radius is much greater than the radius of the tubing bore.

On the basis of these assumptions, the mobile phase is described as laminar flow through a straight circular segment. Arbitrarily, as shown in Figure 2, the heavier phase occupies the peripheral part because of centrifugal force, and the heavier phase is used as the mobile phase, which is represented as the upper part in Figure 2.

According to Figure 2, the retention of the stationary phase (S_f) can be obtained as follows:

$$S_f = \frac{\pi r_c^2 - \left(\frac{1}{2} r_c^2 \varphi - \frac{1}{2} r_c^2 \sin\varphi\right)}{\pi r_c^2} = 1 - \frac{\varphi}{2\pi} + \frac{\sin\varphi}{2\pi} \tag{2}$$

$$\cos\frac{\varphi}{2} = \frac{h}{r_c} \tag{3}$$

where r_c is the radius of the column.

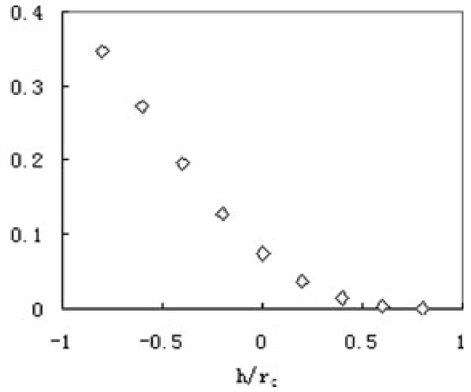


Figure 3. Relationship between *Y* and *h*/r_c.

On the other hand, there exists a relationship between volumetric flow rate F and h/r_c. However, exact analytical solutions for the relationship of the fully developed laminar flow in circular segment ducts are not attainable.[20] Instead, Yu and Sparrow[20] have obtained the numerical results as shown in Figure 3. In which

$$Y = \frac{F}{\left(\frac{r_c^4}{\mu_m}\frac{\Delta P}{L}\right)} \tag{4}$$

Thus, there is an implicit relationship between the volumetric flow rate F and the retention of the stationary phase S_f by using Eqs. 2–4 and Figure 3. Obviously, the implicit relationship is not convenient to use. Therefore, we tried to transform the implicit relationship into an approximate explicit equation. Designate

$$X = (1 - S_f)^2 \tag{5}$$

It is found that Y is approximately proportional to X, as shown in Figure 4.

It is readily shown that in Figure 4:

$$\frac{F}{\left(\frac{r_c^4}{\mu_m}\frac{\Delta P}{L}\right)} = 0.3653(1 - S_f)^2 \tag{6}$$

Thus, the retention of the stationary phase is obtained:

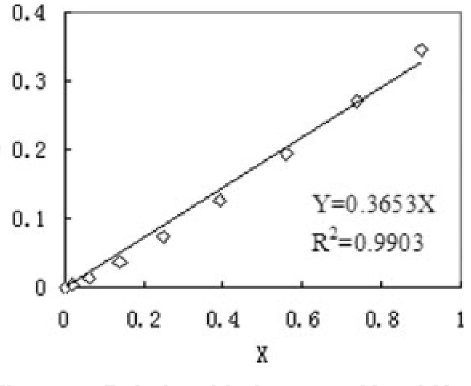


Figure 4. Relationship between *Y* and *X*.

$$S_{\mathrm{f}} = 1 - \frac{6.618}{d^2}\sqrt{\frac{\mu_{\mathrm{m}} L}{\Delta P}}\sqrt{F} \tag{7}$$

Comparing Eq. 7 with Eq. 1, the coefficient in Eq. 7 is only 3.5% lower to that in Eq. 1. The minor difference in the coefficient is mainly due to the different assumption that Eq. 1 is based on the assumption that the cross-section area occupied by the mobile phase can be treated as circular,[14] while Eq. 7 as a segment of a circle, shown in Figure 2. This result also suggests that the Hagen–Poiseuille equation can be applied in this special case of laminar flow.

Equation 7 expresses the relationship between S_{f} and the parameters. Once the pressure drop, ΔP, is known, S_{f} can be predicted. Wood[21] has shown that the pressure drop ΔP of type-J HSCCC is related to the density difference of the phases ($\Delta\rho$), acceleration field ($R\omega^2$), and length (L) of the column, as follows:

$$\Delta P = K\Delta\rho R\omega^2 L \tag{8}$$

Substituting Eq. 8 into Eq. 7, the retention of the stationary phase is obtained as:

$$S_{\mathrm{f}} = 1 - k_1\frac{6.618}{d^2}\sqrt{\frac{\mu_{\mathrm{m}}}{R\Delta\rho}}\frac{\sqrt{F}}{\omega} \tag{9}$$

From Eq. 9, it can be found that the retention of the stationary phase based on laminar flow depends on many parameters such as the column diameter d, the viscosity of the mobile phase μ_{m}, the density difference between the two phases, $\Delta\rho$, the revolution radius R, and also the flow rate F and the rotation speed ω.

Droplet flow

Laminar flow is based on the assumption that the two phases flow as two films along the coil wall. However, when the phases flow as droplets, Equation 9 is no longer valid. According to Fedotov et al.,[18,19] the stationary phase is retained in the column as separate elements having the shape of coarse elongated drops. The stationary phase is usually retained mainly due to a density difference between the mobile and stationary phases, and the Archimedean force is a driving force for the stationary phase retention. In other words, the retention of the stationary phase in droplet flow can be considered as the holdup of the dispersed phase.

The chromatographic column of HSCCC can be considered as a very long extraction column. In extraction columns, there exists the following equation[22]:

$$\frac{F_{\mathrm{c}}}{A_{\mathrm{c}}(1-S_{\mathrm{d}})} + \frac{F_{\mathrm{d}}}{A_{\mathrm{c}}S_{\mathrm{d}}} = (1-S_{\mathrm{d}})\upsilon_0 \tag{10}$$

where S_{d} is the dispersed phase holdup (fractional volume of extraction column occupied by dispersed phase), F_{c} and F_{d} are the flow rates of continuous phase and dispersed phase respectively, and υ_0 is the characteristic droplet velocity.

When $F_{\mathrm{c}} = F$, $F_{\mathrm{d}} = 0$, then $S_{\mathrm{d}} = S_{\mathrm{f}}$, and the retention of the stationary phase can be expressed as:

$$\frac{F}{A_{\mathrm{c}}(1-S_{\mathrm{f}})} = (1-S_{\mathrm{f}})\upsilon_0 \quad \text{or} \tag{11}$$

$$S_{\mathrm{f}} = 1 - \frac{1}{\sqrt{A_{\mathrm{c}}\upsilon_0}}\sqrt{F} \tag{12}$$

Equation 12 agrees well with the finding of a linear relationship between the square root of the flow rate and the retention of the stationary phase.[13] This also validates the viewpoint of Fedotov et al.[18,19] that the stationary phase is retained in the column as separate drops.

In Eq. 12, the characteristic droplet velocity υ_0 defines as the limiting velocity of droplets when $F_{\mathrm{c}} = 0$ and $F_d \to 0$. According to Warshay et al.,[23] very small liquid drops in immiscible liquids behave like rigid spheres. Velocity υ_0 of the liquid drops could be considered as the terminal velocity of solid spheres in a strong centrifugal field, which may obey Stokes law, Allen law, or Newton law depending on the Reynolds number of the liquid drops. Generally speaking, when the Stokes equation is used, the Reynolds number should be substantially less than unity. Because of the heterogeneous centrifugal force field and small column diameter in HSCCC, the liquid drops are usually very small, and the terminal velocity is approximately considered as having reached the equilibrium velocity. As a result it could be assumed that velocity υ_0 obeys the Stokes law that defines v_0, the velocity of a hard sphere of diameter d_{p}, in a liquid system of viscosity, μ_{m}, submitted to a roughly estimated centrifugal field,[24] $R\omega^2$. Therefore, the velocity υ_0 is:

$$\upsilon_0 = \frac{d_{\mathrm{p}}^2\Delta\rho\omega^2 R}{18\mu_{\mathrm{m}}} \tag{13}$$

where d_{p} is the droplet diameter.

Equation 12 then becomes:

$$S_{\mathrm{f}} = 1 - \frac{6}{d\cdot d_{\mathrm{p}}}\sqrt{\frac{2\mu_{\mathrm{m}}}{\pi\Delta\rho R}}\frac{\sqrt{F}}{\omega} \tag{14}$$

The droplet diameter was correlated using dimensional analysis,[25] assuming that droplet diameter is a function of several variables:

$$d_{\mathrm{p}} = d_{\mathrm{p}}(\mu_{\mathrm{m}}, \Delta\rho, \sigma, d) \tag{15}$$

The following dimensionless correlation was found:

$$d_{\mathrm{p}} = a\left(\frac{(\Delta\rho\sigma d)^{1/2}}{\mu_{\mathrm{m}}}\right)^{b} d = a\, Oh^{-b}\, d \tag{16}$$

where a and b are constants and Oh is the Ohnesorge number, defined as:

$$Oh = \frac{\mu_{\mathrm{m}}}{(\Delta\rho d\sigma)^{1/2}} = \frac{(We)^{1/2}}{\mathrm{Re}} \tag{17}$$

Ohnesorge number is a ratio of the viscous forces and the square root of the product of inertia and interfacial tension forces, and it is also the ratio of the square root of Weber number (*We*) and Reynolds number (*Re*). From Eqs. 14, 16, and 17, we can obtain:

$$S_{\mathrm{f}} = 1 - \frac{k_2}{d^2}\left(\frac{\mu_{\mathrm{m}}}{(\Delta\rho\sigma d)^{1/2}}\right)^{c}\sqrt{\frac{\mu_{\mathrm{m}}}{\Delta\rho R}}\frac{\sqrt{F}}{\omega} \tag{18}$$

According to the finding of Berthod and Schmitt[7] the retention of the stationary phase is directly proportional to the density difference between the two liquid phases, the constant c in Eq. 18 equals to 1. Therefore, the retention of the stationary phase is obtained as:

$$S_{\mathrm{f}} = 1 - \frac{k_2}{d^2}\left(\frac{\mu_{\mathrm{m}}}{(\Delta\rho\sigma d)^{1/2}}\right)\sqrt{\frac{\mu_{\mathrm{m}}}{\Delta\rho R}}\frac{\sqrt{F}}{\omega} = 1 - \frac{k_2}{d^2}Oh\sqrt{\frac{\mu_{\mathrm{m}}}{\Delta\rho R}}\frac{\sqrt{F}}{\omega} \tag{19}$$

From Eq. 19, it can be found that S_{f} based on droplet flow not only depends on the parameters that are also in the laminar flow model such as the column diameter d, the viscosity of the mobile phase μ_{m}, the density difference between the two phases $\Delta\rho$, the revolution radius R, the flow rate F and the rotation speed ω, but also on the interfacial tension σ.

From Eqs. 9 and 19, it can be easily found that β-value was not included. The reason may be as follows: (1) Influence of β-value on the retention of the stationary phase mainly lies in whether the stationary phase can be retained well or not. When β is greater than 0.25, a unilateral distribution of the two phases occurs. HSCCC has been developed by utilizing this unique centrifugal force field. When β-value is further increased up to 0.5, the stationary phase can be retained well. So the β-value is recommended higher than 0.6.[4] (2) The standard commercial HSCCC usually has a β-value from 0.5 to 0.8.

Comparing Eq. 19 with Eq. 9, it is found that the relationship between the retention of the stationary phase and the flow rate and rotation speed is similar no matter whether the flow is laminar or droplet, as follows:

$$1 - S_{\mathrm{f}} \propto \frac{\sqrt{F}}{\omega} \tag{20}$$

If we designate YSF as:

$$\mathrm{YSF} = \frac{(1 - S_{\mathrm{f}})d^2}{\sqrt{\frac{\mu_{\mathrm{m}}}{R\Delta\rho}}\sqrt{F}/\omega} \tag{21}$$

then

$$\mathrm{YSF} = \begin{cases} 6.618k_1 & \text{laminar flow} \\ k_2\left(\frac{\mu_{\mathrm{m}}}{(\Delta\rho\sigma d)^{1/2}}\right) = k_2\,Oh & \text{droplet flow} \end{cases} \tag{22}$$

The prediction model based on the laminar flow and droplet flow includes two unknown parameters k_1 and k_2, which need to be determined by correlation with the experimental data. Therefore, the experiments are performed with varying the solvent systems and operation conditions.

Experimental

Apparatus

The HSCCC instrument employed in this paper is a preparative Model TBE-1000A high-speed countercurrent chromatography (Tauto Biotech, Shanghai, China) equipped with a 1000-ml coil column made of polytetrafluorethylene (PTFE) tubing (3.0 mm) and a 100-ml sample loop. It contains three multilayer connected in series spinning with a planetary motion around a central axis. The type J multilayer coil planet centrifuge with a 13-cm revolution radius produces a synchronous planetary motion of the column holder. The β-value of the preparative column varied from 0.59 at the internal layer to 0.75 at the external layer. The rotation speed of the apparatus can be regulated with a speed controller in the range between 0 and 600 rpm. The HSCCC system is equipped with a Model SD-9002 constant-flow pump, a HX-2050 water bath, a Model 8823B UV monitor and a Model N2010 chromatography workstation.

Two-phase solvent systems

N-hexane-ethyl acetate-ethanol-water system is a universal two-phase solvent system which provides a broad range of hydrophobicity, and is extremely useful for separations of various natural products. Table 1 gives 10 selected two-phase solvent systems, which include binary, ternary and quaternary systems, and have a broad range of interfacial tension (0.5–50 mN/m) and the density difference between the two phases (90–340 kg/m^3). S_{f} values of the ten two-phase solvent systems (Table 1) are used to correlate the model parameters.

Some more polar or low interfacial tension two-phase solvent systems, such as *n*-butanol-water, *n*-butanol-acetic acid-water (4:1:5, v/v), *n*-hexane-methanol, etc, are quite useful for separation of biological samples (butanol systems) and nonpolar labile samples requiring nonaqueous solvent system (hexane–methanol system). Therefore, as shown in Table 2, the six selected two-phase solvent systems including binary, ternary, and quaternary systems, which differ from those systems used in correlating model parameters in Table 1, are also considered. S_{f} values of the six two-phase solvent systems (Table 2) are used to test the model.

Procedure

The two-phase solvent systems were prepared by adding all the solvents to a separation funnel according to the volume ratios and thoroughly equilibrated by shaking repeatedly. The upper phase and the lower phase were separated for 30 min prior to use.

The retention of the stationary phase (S_{f}) was determined by the following procedure. The coiled column was first entirely filled with the stationary phase, and then the appara-

Table 1. Two-Phase Solvent Systems for Correlating the Model Parameters

Number	Two-Phase Solvent System
1a	Hexane-water (10:10, v/v)
2a	Ethyl acetate-water (10:10, v/v)
3a	Hexane-ethyl acetate-water (2:8:10, v/v)
4a	Hexane-ethyl acetate-water (5:5:10, v/v)
5a	Hexane-ethyl acetate-water (8:2:10, v/v)
6a	Hexane-ethyl acetate-ethanol-water (1:5:1:5, v/v)
7a	Hexane-ethyl acetate-ethanol-water (3:5:3:5, v/v)
8a	Hexane-ethyl acetate-ethanol-water (5:5:5:5, v/v)
9a	Hexane-ethyl acetate-ethanol-water (8:2:5:5, v/v)
10a	Hexane-ethyl acetate-ethanol-water (8:2:8:2, v/v)

Table 2. Two-Phase Solvent Systems for Testifying the Model

Number	Two-Phase Solvent System
11a	Hexane-methanol (10:10, v/v)
12a	Heptane-methanol (10:10, v/v)
13a	Ethyl acetate-*n*-butanol-water (3:2:5, v/v)
14a	*N*-butanol-water (10:10, v/v)
15a	*N*-butanol-acetic acid-water (4:1:5, v/v)
16a	Hexane-ethyl acetate-methanol water (1:9:1:9, v/v)

tus was rotated at a given rotation speed, while the mobile phase was pumped into the column at a desired flow rate. After the mobile phase front emerged and hydrodynamic equilibrium was established in the column, the volume of the stationary phase from the column was measured. Then, the volume of mobile phase in the column could be calculated by subtracting the volume of inlet and outlet leads, and the retention of the stationary phase could be calculated.[26]

Results and Discussion

The experimental data of the retention of the stationary phase at different solvent systems and operation conditions are shown in Appendix.

Influence of flow rate and rotation speed on the retention of the stationary phase

In HSCCC, flow rate and rotation speed have great influence on the retention of the stationary phase. As shown in Appendix, for a given two-phase solvent system, the retention of the stationary phase increases with the rotation speed and decreases with the flow rate. Furthermore, using the data in Appendix, it is found that the retention of the stationary phase decreases proportionally to the ratio of the square root of the mobile phase flow rate to the rotation speed ($F^{1/2}/\omega$) as shown in Figure 5, where the systems 1a-10a are given in Table 1. With a regression analysis of $S_f = A - B\sqrt{F}/\omega$, it is found that the correlation coefficients are between 0.96 and 0.99, and mean correlation coefficient is 0.98. In most cases, A value nearly equals to 1. This result validates Eq. 20. However, we can also find that some curves agree worse than other, such as system 2a (ethyl acetate-water). When the ideal unilateral hydrodynamic distribution is obtained, that is, one phase entirely occupies the head side and the other phase, the tail side of the coil. Under this ideal condition, A value equals to 1. But it is not always possible to completely place 0% heavy phase at one end and 100% at the other.[27] This is very dependent on the physical properties of the two-phase solvent system. High interfacial tension with large density difference and low viscosity phase systems would distribute in an ideal way. But for some low interfacial tension, low phase density difference and high viscosity systems, the ideal way would not always be guaranteed. For system ethyl acetate -water, it is mainly due to the relatively lower density difference between the two phases.

Furthermore, the linear relationship between S_f and $F^{1/2}/\omega$ also verifies the assumption that the characteristic droplet velocity υ_0 obeys the Stokes law.

Influence of physical properties of two-phase solvent systems

It is well-known that physical properties of the two-phase system have great influences on the retention of the stationary phase. According to Eqs. 9 and 19, S_f increases with the density difference and decreases with the viscosity of the mobile phase. However, the influence of the interfacial tension is different. In the laminar flow model, the interfacial tension has no influence, while in the droplet flow model, S_f increases with the interfacial tension. To provide a more quantitative insight of the physical properties of the two-phase solvent system, a three-dimensional figure, Figure 6, was constructed using the data in Appendix. Figure 6 shows the relationship between the value of "YSF" (defined in Eq. 21) and Eo number (Eötvös number) and Oh number. Eötvös number is the ratio of the centrifugal forces to the interfacial tension forces, and is defined as:

$$Eo = \frac{\Delta\rho\omega^2 Rd^2}{\sigma} = \frac{\text{Centrifugal force}}{\text{Interfacial tension force}} \tag{23}$$

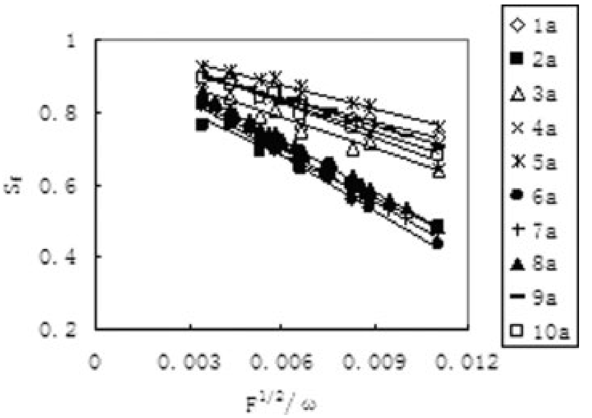


Figure 5. Relationship between retention of the stationary phase and $F^{1/2}/\omega$.

(Flow rate F: 3–11 ml/min; rotation speed ω: 300–500 rpm).

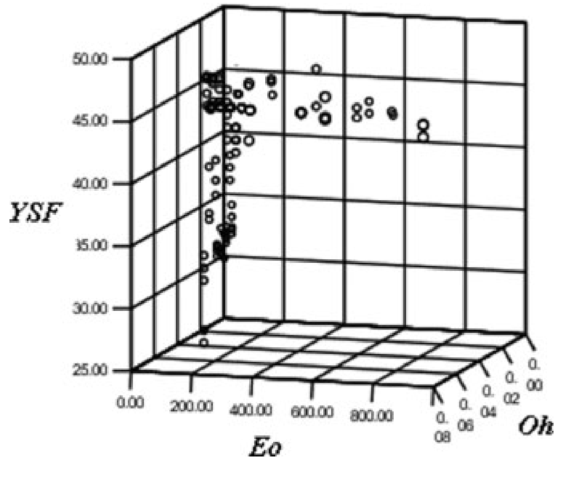


Figure 6. Relationship between *Eo*, *Oh*, and *YSF*.

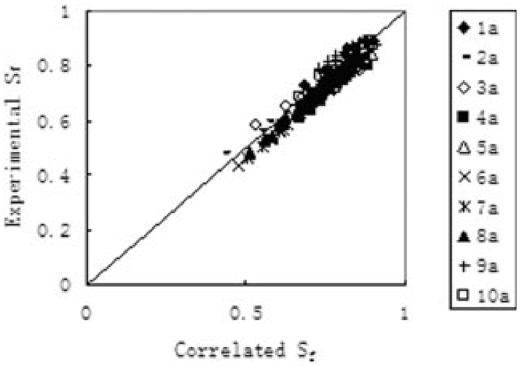


Figure 7. Correlation results using Eq. 25 for the solvent systems in Table 1.

(Flow rate: 3–11 ml/min; rotation speed: 300–500 rpm, density difference between the two phases: 90–340 kg/m^3, interfacial tension: 0.5–50 mN/m).

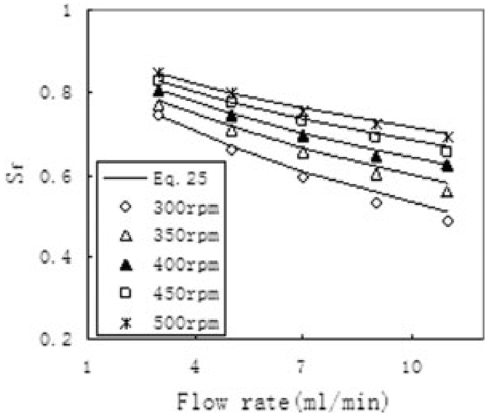


Figure 8. Correlation results for the two-phase solvent system of *n*-hexane-ethyl acetate-ethanol-water (1:1:1:1, v/v).

(Flow rate: 3–11 ml/min; rotation speed: 300–500 rpm).

The physical properties of the two-phase solvent systems (μ_m, $\Delta\rho$, σ) are calculated using the program[28] developed by Zhang and co workers.

From Eq. 22, "YSF" has two different kinds of values corresponding to two different flow regimes. When the flow regime in the coiled column is laminar flow, "YSF" keeps constant as $6.618k_1$; and when the flow regime is droplet flow, "YSF" has an approximately linear relationship with the *Oh* number. From Figure 6, it can be easily found that two separate regions are distinguished, where one is that "YSF" approximately equals to 44, the other is that "YSF" has a linear relationship with *Oh* number, and the slope is 1500. Then the model parameters k_1 and k_2 can be determined as: $k_1 = 6.649$, and $k_2 = 1500$. Then the prediction model of S_f is obtained as:

$$\text{Laminar flow model:} \quad S_f = 1 - \frac{44}{d^2}\sqrt{\frac{\mu_m}{\Delta\rho R}}\frac{\sqrt{F}}{\omega} \tag{24a}$$

Droplet flow model:

$$S_f = 1 - \frac{1500}{d^2}\left(\frac{\mu_m}{(\Delta\rho\sigma d)^{1/2}}\right)\sqrt{\frac{\mu_m}{\Delta\rho R}}\frac{\sqrt{F}}{\omega} \tag{24b}$$

Transition of the laminar flow and droplet flow

The model of retention of the stationary phase depends on two flow regimes: the laminar flow and droplet flow. It is important to determine the transition from the laminar flow to the droplet flow. As shown in Figure 6, the critical value at which the transition between the laminar flow and droplet flow occurs is dependent on the dimensionless number *Eo*. When the *Eo* number is higher than 80, which means that the centrifugal force is much larger than the interfacial tension force and the interfacial tension force can be neglected, then the prediction of S_f is based on the laminar flow model (Eq. 24a). When the *Eo* number is lower than 80, that is the interfacial tension force is large enough and can not be ignored, then the droplet flow model (Eq. 24b) is best for the prediction of S_f. It should be noted that, if the two-phase solvent system has a very high interfacial tension, for example above 10 mN/m according to the calculation using the program,[28] such as the binary systems of *n*-hexane-water and of ethyl acetate-water, the laminar flow model is recommended, for the system tends to separate as two layers and flow as laminar flow. Therefore, the prediction model of the retention of the stationary phase is obtained as:

$$S_f = \begin{cases} 1 - \frac{44}{d^2}\sqrt{\frac{\mu_m}{\Delta\rho R}}\frac{\sqrt{F}}{\omega} & Eo > 80 \text{ or } \sigma > 10 \\ 1 - \frac{1500}{d^2}\left(\frac{\mu_m}{(\Delta\rho\sigma d)^{1/2}}\right)\sqrt{\frac{\mu_m}{\Delta\rho R}}\frac{\sqrt{F}}{\omega} & Eo < 80 \end{cases} \tag{25}$$

Correlation results of the model

Equation 25 is used to correlate the S_f values (Appendix) of the solvent systems in Table 1. For the solvent systems of 1a-8a in Table 1, the laminar flow model is adopted. For the systems of 9a and 10a, the droplet flow model is used due to the lower *Eo* number.

The comparison of the correlated and experimental retention of the stationary phase is shown in Figure 7. As a typi-

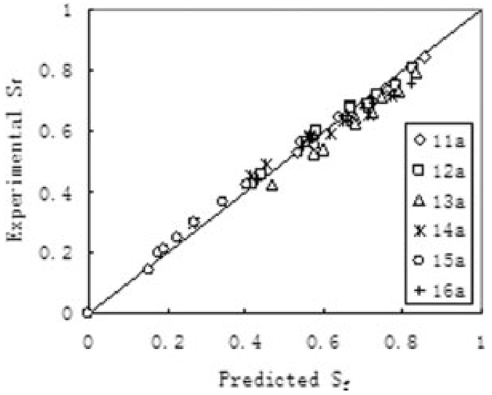


Figure 9. Prediction results using Eq. 25 comparing with the experimental data (Table 2).

(Flow rate: 1–11 ml/min; rotation speed: 300–550 rpm).

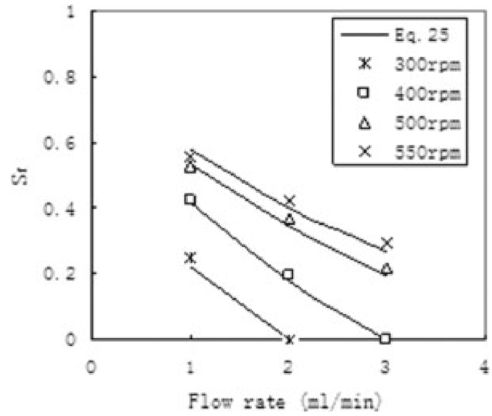


Figure 10. Prediction results using Eq. 25 for the two-phase solvent system of *n*-butanol-acetic acid-water (4:1:5, v/v).

(Flow rate: 1–3 ml/min; rotation speed: 300–550 rpm).

cal example, the detailed comparison of the solvent system *n*-hexane-ethyl acetate-ethanol-water (1:1:1:1, v/v) is shown in Figure 8, compared with the laminar flow model prediction. Figures 7 and 8 showed that the model could correlate the retention of the stationary phase well. The total average absolute deviation (AAD%) for 10 solvent systems and 120 data points is 3.79%, and the maximum deviation is 10.4%.

Prediction results of the model

Prediction Results of the Model Comparing to the Experimental Data in this Work Equation 25 is tested with the solvent systems in Table 2. For the solvent systems of 12a-14a and 16a, the laminar flow model is adopted. For the systems of 11a and 15a, the droplet flow model is used due to the lower *Eo* number. The comparison of the predicted and experimental retention of the stationary phase is shown in Figure 9. Figure 9 shows that the model can predict the retention of the stationary phase well. The total average absolute deviation (AAD%) for the six solvent systems and 56 data points is 4.76%, and the maximum deviation is 10.6%.

As a typical example, the detailed comparison of the solvent system *n*-butanol-acetic acid-water (4:1:5, v/v) is shown in Figure 10, compared with the droplet flow model prediction. It should be noted that, when the rotation speed is 300 rpm and the flow rate reaches 2 ml/min, or the rotation speed 400 rpm and the flow rate 3 ml/min, the predicted S_f value is negative, which means the retention of the stationary phase is zero. This prediction result agrees well with the experimental data that the stationary phase can not be retained under these operation conditions, as shown in Appendix.

Prediction Results of the Model Comparing to Literature Data. Equation 25 is used to predict the retention of the stationary phase of all the solvent systems in Table 3 (literature data). The comparison of the predicted and literature data[13,14,29–31] is shown in Figure 11.

In Figure 11, the *Eo* numbers of the two-phase solvent systems of 1b-9b and 15b are larger than 80, and the interfacial tensions of the binary systems of 12b and 13b are larger than 10 mN/m, so the laminar flow model is adopted to predict the retention of the stationary phase. For the solvent systems of 10b, 11b, 14b, and 16b, the *Eo* numbers are smaller than 80, the droplet flow model is adopted to predict the retention of the stationary phase. It was shown in Figure 11 that the predicted retentions of the stationary phase are in

Table 3. Data Sources and Prediction Results

Apparatus Type	Author, Data Sources, and Data Points (N)		Solvent System	AAD (%)	Max (%)
Preparative	Sutherland[31] ($V_c = 928$ ml, $d = 3.68$ mm, $\beta = 0.63$–0.87), $N = 4$	1b	Heptane-ethyl acetate-methanol-water (1.4:0.1:0.5:1.0, v/v)	6.56	7.80
Semi-preparative	Du[13] ($V_c = 230$ ml, $d = 1.6$ mm, $\beta = 0.4$–0.78), $N = 62$	2b	Hexane-ethyl acetate-water (1:9:10, v/v)		
		3b	Hexane-ethyl acetate-water (2:8:10, v/v)		
		4b	Hexane-ethyl acetate-water (5:5:10, v/v)		
		5b	Hexane-ethyl acetate-water (8:2:10, v/v)		
		6b	Hexane-ethyl acetate-methanol-water (1:9:1:9, v/v)		
		7b	Hexane-ethyl acetate-methanol-water (2:8:2:8, v/v)		
		8b	Hexane-ethyl acetate-methanol-water (4:6:4:6, v/v)		
		9b	Hexane-ethyl acetate-methanol-water (5:5:5:5, v/v)		
		10b	Hexane-ethyl acetate-methanol-water (6:4:6:4, v/v)	2.80	12.8
		11b	Hexane-ethyl acetate-methanol-water (8:2:8:2, v/v)		
		12b	Hexane-water (10:10, v/v)		
		13b	Ethyl acetate-water (10:10, v/v)		
	Berthod[29] ($V_c = 156$ ml, $d = 1.65$ mm, $\beta = 0.56$), N=15	14b	Heptane-methanol-water (10: 9:1, v/v)		
	Wood[14]($V_c = 59.1$, 120.5, 259.5 ml, $d = 3.73$, 5.33, 7.73 mm, $\beta = 0.95$), $N = 65$	15b	Heptane-ethyl acetate-methanol-water (1.0:0.1:0.5:1.0, v/v)		
Analytical	Sutherland[30] ($V_c = 4.6$ ml, $d = 0.76$ mm, $\beta = 0.68$–0.79), $N = 5$	16b	Heptane-ethyl acetate-methanol-water (1.4:0.6:1.0:1.0, v/v)	4.77	7.87
Total	$N = 151$		16	2.96	12.8

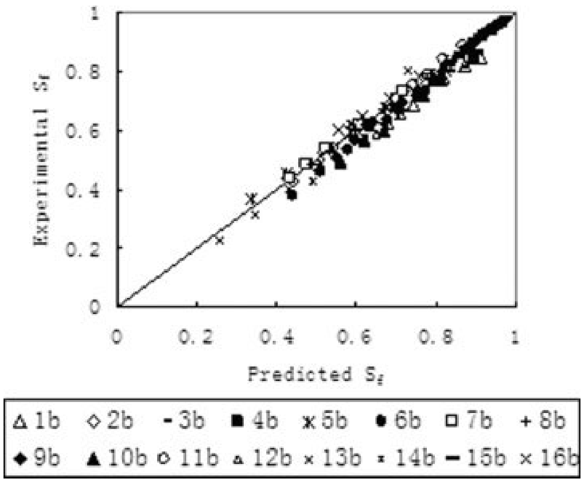


Figure 11. Prediction results using Eq. 25 comparing to the literature data in Table 3.

(Flow rate: 0.8–80 ml/min; rotation speed: 500–1800 rpm; density difference between the two phases: 90–300 kg/m^3; interfacial tension: 0.2–50 mN/m).

good agreement with the literature S_f. The total average absolute deviation (AAD%) for 16 solvent systems and 151 data points is 2.96%, and the maximum deviation is 12.8%. As a typical example, the detailed comparison for the solvent systems of 2b-4b is shown in Figure 12.

The prediction covers three kinds of J-type HSCCC apparatus including analytical, semi-preparative, and preparative HSCCC. Furthermore, there are different tube materials including PTFE and stainless steel with ID coils between 0.76 and 7.73 mm and β values between 0.4 and 0.95. Thus the prediction results of the model are satisfactory.

Conclusions

A mathematical model is developed to predict the retention of the stationary phase (S_f) based on the flow behavior of the two phases in the coiled column, laminar flow or droplet flow. The model shows that S_f decreases linearly with the square root of the flow rate, and increases with rotation speed, and there is a linear relationship between S_f and $F^{1/2}/\omega$. The critical value at which the transition between the laminar flow and droplet flow occurs is dependent on the dimensionless number *Eo*. The model agrees well with the experimental data of retention of the stationary phase at different two-phase solvent systems and operation conditions measured in this work. Furthermore, the model can well predict the literature data of retention of the stationary phase for three types of HSCCC apparatus including preparative, semi-preparative, and analytical with two different tube materials of PTFE and stainless steel. The total average absolute deviation (AAD%) predicted for 16 solvent systems and 151 data points is 2.96%, and the maximum deviation is 12.8%.

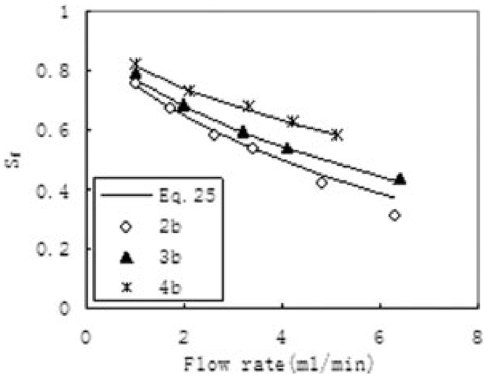


Figure 12. Prediction results using Eq. 25 for the two-phase solvent systems 2b-4b in Table 3.

(Flow rate: 1–7 ml/min; rotation speed: 750 rpm).

The model can be used to predict the retention of the stationary phase at different operation conditions (flow rate, rotation speed), physical properties (density difference, viscosity and interfacial tension), and instrument parameters (tube diameter, revolution radius). It is helpful for the design and scaling-up of high-speed countercurrent chromatography.

Acknowledgments

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Notation

a = parameter in Eq. 16
A_c = cross-sectional area of the CCC column (m^2)
b = parameter in Eq. 16
c = parameter in Eq. 18
d = internal diameter of the tube (m)
d_p = droplet diameter (m)
Eo = Eötvös number
F = flow rate of the mobile phase (m^3/s or ml/min)
F_c = flow rate of continuous phase (m^3/s)
F_d = flow rate of dispersed phase (m^3/s)
h = location of interface (m)
k_1 = parameter in Eq. 9
k_2 = parameter in Eq. 18
L = the total length of the tube (m)
m = layers of the coil
n = turns of tube
N = data points
Oh = ohnesorge number
ΔP = pressure drop (Pa)
r = coil radius (m)
r_c = radius of the tube (m)
R = revolution radius (m)
S_d = dispersed phase hold up
S_f = retention of stationary phase
v_0 = characteristic droplet velocity (m/s)
V_c = total column volume (ml)
V_s = volume of stationary phase (ml)
X = defined by Eq. 5
Y = defined by Eq. 4
YSF = defined by Eq. 21

Greek letters

μ = viscosity (Pa·s)
ω = rotation speed (rad/s)
σ = interfacial tension (N/m)

$\Delta\rho$ = density difference between the lower phase and upper phase (kg/m^3)
β = the ratio of the planetary radius to the rotor radius (r/R)
φ = angle in Fig. 2

Subscripts

m = mobile phase
s = stationary phase
c = continuous phase
d = dispersed phase

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Appendix

Table A1. Retention of the Stationary Phase at Different Systems and Operation Conditions

ω (rpm)	F (ml/min)	S_f
Hexane-water (10:10, v/v)		
300	3	0.863
300	7	0.782
300	11	0.73
400	3	0.886
400	7	0.836
400	11	0.78
500	3	0.892
500	7	0.843
500	11	0.816
Ethyl acetate-water (10:10, v/v)		
300	3	0.717
300	7	0.568
300	11	0.480
400	3	0.770
400	7	0.645
400	11	0.599
500	3	0.765
500	7	0.696
500	11	0.647
Hexane-ethyl acetate-water (2:8:10, v/v)		
300	3	0.807
300	7	0.718
300	11	0.642
400	3	0.844
400	7	0.770
400	11	0.701
500	3	0.859
500	7	0.789
500	11	0.749

Table A1. (*Continued*)

ω (rpm)	F (ml/min)	S_f
Hexane-ethyl acetate-water (5:5:10, v/v)		
300	3	0.765
300	7	0.672
300	11	0.582
400	3	0.802
400	7	0.744
400	11	0.702
500	3	0.804
500	7	0.762
500	11	0.735
Hexane-ethyl acetate-water (8:2:10, v/v)		
300	3	0.896
300	7	0.816
300	11	0.756
400	3	0.912
400	7	0.867
400	11	0.823
500	3	0.925
500	7	0.885
500	11	0.851
Hexane-ethyl acetate-ethanol-water (1:5:1:5, v/v)		
300	3	0.694
300	7	0.533
300	11	0.436
400	3	0.771
400	7	0.650
400	11	0.561
500	3	0.817
500	7	0.720
500	11	0.649
Hexane-ethyl acetate-ethanol-water (3:5:3:5, v/v)		
300	3	0.708
300	5	0.624
300	7	0.555
300	9	0.505
300	11	0.464
350	3	0.750
350	5	0.677
350	7	0.618
350	9	0.567
350	11	0.532
400	3	0.781
400	5	0.718
400	7	0.666
400	9	0.622
400	11	0.582
450	3	0.806
450	5	0.749
450	7	0.704
450	9	0.664
450	11	0.628
500	3	0.825
500	5	0.775
500	7	0.733
500	9	0.697
500	11	0.666
Hexane-ethyl acetate-ethanol-water (5:5:5:5, v/v)		
300	3	0.741
300	5	0.659
300	7	0.591
300	9	0.533
300	11	0.485
350	3	0.769
350	5	0.707
350	7	0.656
350	9	0.601
350	11	0.557
400	3	0.804
400	5	0.742
400	7	0.694
400	9	0.648
400	11	0.623
450	3	0.829
450	5	0.776
450	7	0.729
450	9	0.688
450	11	0.655
500	3	0.845
500	5	0.796
500	7	0.753
500	9	0.721
500	11	0.689
Hexane-ethyl acetate-ethanol-water (8:2:5:5, v/v)		
300	3	0.848
300	5	0.809
300	7	0.770
300	9	0.737
300	11	0.707
350	3	0.863
350	5	0.833
350	7	0.805
350	9	0.778
350	11	0.754
400	3	0.880
400	5	0.849
400	7	0.824
400	9	0.799
400	11	0.776
450	3	0.887
450	5	0.862
450	7	0.838
450	9	0.818
450	11	0.791
500	3	0.894
500	5	0.870
500	7	0.847
500	9	0.826
500	11	0.804
Hexane-ethyl acetate-ethanol-water (8:2:8:2, v/v)		
300	3	0.856
300	7	0.763
300	11	0.684
400	3	0.876
400	7	0.816
400	11	0.759
500	3	0.893
500	7	0.843
500	11	0.795
Hexane-methanol (1:1, v/v)		
300	3	0.731
300	7	0.643
300	11	0.564
400	3	0.806
400	7	0.716
400	11	0.646
500	3	0.842
500	7	0.758
500	11	0.697
Heptane-methanol (10:10, v/v)		
300	3	0.689
300	7	0.566
300	11	0.462
400	3	0.751
400	7	0.682
400	11	0.604
500	3	0.808
500	7	0.722
500	11	0.674

Table A1. (*Continued*)

ω (rpm)	F (ml/min)	S_f
Ethyl acetate-*n*-Butanol-water (3:2:5, v/v)		
300	3	0.665
300	7	0.531
300	11	0.42
400	3	0.735
400	7	0.646
400	11	0.542
500	3	0.796
500	7	0.712
500	11	0.628
Butanol-water (1:1, v/v)		
300	3	0.593
300	7	0.456
300	11	0.299
400	3	0.655
400	7	0.577
400	11	0.493
500	3	0.713
500	7	0.635
500	11	0.581
N-butanol-acetic acid-water (4:1:5, v/v)		
300	1	0.251
300	2	0
400	1	0.421
300	11	0.42
400	3	0.735
400	7	0.646
400	11	0.542
500	3	0.796
500	7	0.712
500	11	0.628
Butanol-water (1:1, v/v)		
300	3	0.593
300	7	0.456
300	11	0.299
400	3	0.655
400	7	0.577
400	11	0.493
500	3	0.713
500	7	0.635
500	11	0.581
N-butanol-acetic acid-water (4:1:5, v/v)		
300	1	0.251
300	2	0
400	1	0.421
400	2	0.196
400	3	0
500	1	0.530
500	2	0.365
500	3	0.214
550	1	0.555
550	2	0.422
550	3	0.296
Hexane-ethyl acetate-methanol water (1:9:1:9, v/v)		
300	3	0.674
300	7	0.551
300	11	0.442
400	3	0.721
400	7	0.634
400	11	0.575
500	3	0.760
500	7	0.698
500	11	0.651

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