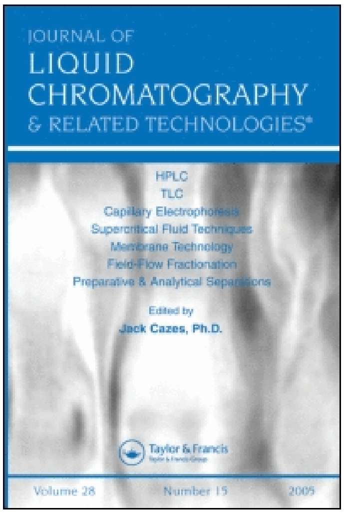

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Centrifugal Partition Chromatography. II. Selectivity Efficiency

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CENTRIFUGAL PARTITION CHROMATOGRAPHY. II. SELECTIVITY AND EFFICIENCY

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ABSTRACT

Centrifugal Partition Chromatography (CPC) is a technique that uses two immiscible liquids. One liquid is used as a stationary phase, the second one as the mobile phase. Using the liquid systems methanol-hexane and octanol-water, the selectivity and efficiency of CPC apparatus were tested. It is shown that CPC can be used to determine partition coefficients or to purify compounds. The selectivity can be changed the same way as in liquid chromatography. The efficiency study has shown that the plate count presents a minimum value at a particular flow rate. By plotting the number of channels required to obtain one theoretical plate versus the flow rate, it is possible to obtain a "van Deemter-type" plot. Interestingly, these plots show maxima for CPC. These results are opposite to those found for liquid or gas chromatography which have minima. A flow change, from laminar to nonlaminar is thought to explain these results. Reynolds numbers were determined for all solvent systems. Efficiency in CPC was also found to be solute dependent: the larger the partition coefficient, the lower the efficiency.

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INTRODUCTION

In the previous paper of this series the general features of Centrifugal Partition Chromatography (CPC) were presented (1). CPC is a variation on counter current chromatography (CCC) (2,3). As in CCC, two immiscible liquids are used. The first liquid is the stationary phase, the second is the mobile phase. The liquid stationary phase is held in channels engraved in several Polychlorotrifluoroethylene (PCTFE) plates. One hundred channels are engraved on each PCTFE plate. Four PCTFE plates are assembled together in a cartridge. Up to 12 cartridges (4800 channels) can be loaded in the rotor of a centrifuge. The centrifugal field, generated by the spinning rotor holds the stationary phase sufficiently tight that a mobile phase may be pumped through it. We have shown that the driving pressure depends on the spin and flow rates, on the physico-chemical properties of the liquid systems (viscosity and density) and on the amount of stationary phase entrapped in the apparatus by the centrifugal field.

The aim of the present work is to study the analytical figures of merit of CPC. Namely, the selectivity, or ability to separate two compounds, and the efficiency, or mass transfer between phases, will be evaluated.

EXPERIMENTAL SECTION

1-Apparatus

The CPC apparatus was model CPC-NMF from Sanki (Sanki Laboratories Inc., Sharon Hill, PA) and was fully described in the previous paper of this series (1). The rotor was loaded with six cartridges, type 250 W, each containing 400 channels. The total volume was approximately 125 mL, with 2400 total channels. A LC pump (Shimadzu, model LC-6A) and a

UV-vis detector (Shimadzu, Model SPD-6A, with a preparative flow cell) were connected to the CPC system to circulate the liquids and to detect the solutes, respectively.

2-Liquid Systems

Two very different liquid systems were used. The first system was methanol-hexane. Those two liquids have low densities (0.791 and 0.661 g/cm^3 at 20^{o}C, respectively), low viscosities (0.54 and 0.29 cP at 25^{o}C, respectively) and have a mutual solubility sufficiently high that it was necessary to add 1% v/v of water in methanol to decrease the hexane solubility. The added water also served to increase the interfacial tension between the two liquid phases. The second liquid system was octanol-water. Octanol has a high viscosity (3.6 cP at 25^{o}C) and a relatively low density (0.845 g/cm^3 at 25^{o}C). The first system was chosen for its convenience to handle: the phase saturation process is fast and the two phases have relatively similar polarities which produces low values for the partition coefficients of a variety of solutes. The second system was chosen because it is the reference system for the study of the "hydrophobic bonding" of active drugs in biochemistry and pharmaceutical chemistry (4).

The physico-chemical properties of the equilibrated phases (one liquid saturated with the other) are quite different from those of the pure liquids. These properties and their variation is the subject of paper number three in this series (5).

The solutes used in this study were 1-naphthol (from Fisher), DDT (2,2-p-chlorophenyl-1,1,1-trichloroethane), DDD (1,1-p-chlorophenyl-2,2-dichloroethane) (Aldrich), pentachlorophenol and hydroquinone (Sigma). All experiments were performed at 25^{o}C, the rotor of the CPC apparatus being enclosed in a constant temperature box.

RESULTS AND DISCUSSION

CPC can be used to separate compounds or to determine partition coefficients. As in any other chromatographic technique, the separation and/or purification process is dependent on two parameters: selectivity and efficiency. The partition coefficient determination depends mainly on selectivity. In the separation of two compounds 1 and 2, a good selectivity will produce two very different retention times. A high efficiency will produce narrow peaks. The peak resolution, R_s, is expressed as the ratio of the distance between the two peak maxima to the mean value of the peak width, W, at the baseline.

$$R_s = (t_2 - t_1) / [(W_2 + W_1 / 2] \qquad [1]$$

The numerator of eq. 1 only depends on selectivity, the denominator depends on efficiency. Both parameters are evaluated in the following paragraphs.

1-Selectivity

As shown in the previous paper of this series (1), the basic retention equation in CPC is expressed by:

$$v_r = v_t + (K - 1) v_s \qquad [2]$$

in which: v_r is the retention volume of the compound (mL),
v_t is the total internal volume of the CPC apparatus (mL),
v_s is the stationary phase volume (mL),
and K is the partition coefficient of the compound between the stationary and mobile phase.

Figure 1A is a 3D-plot of eq. 2 within the conditions of our experiments, i.e., v_t = 125 mL with six cartridges. It can be seen that: (i) for

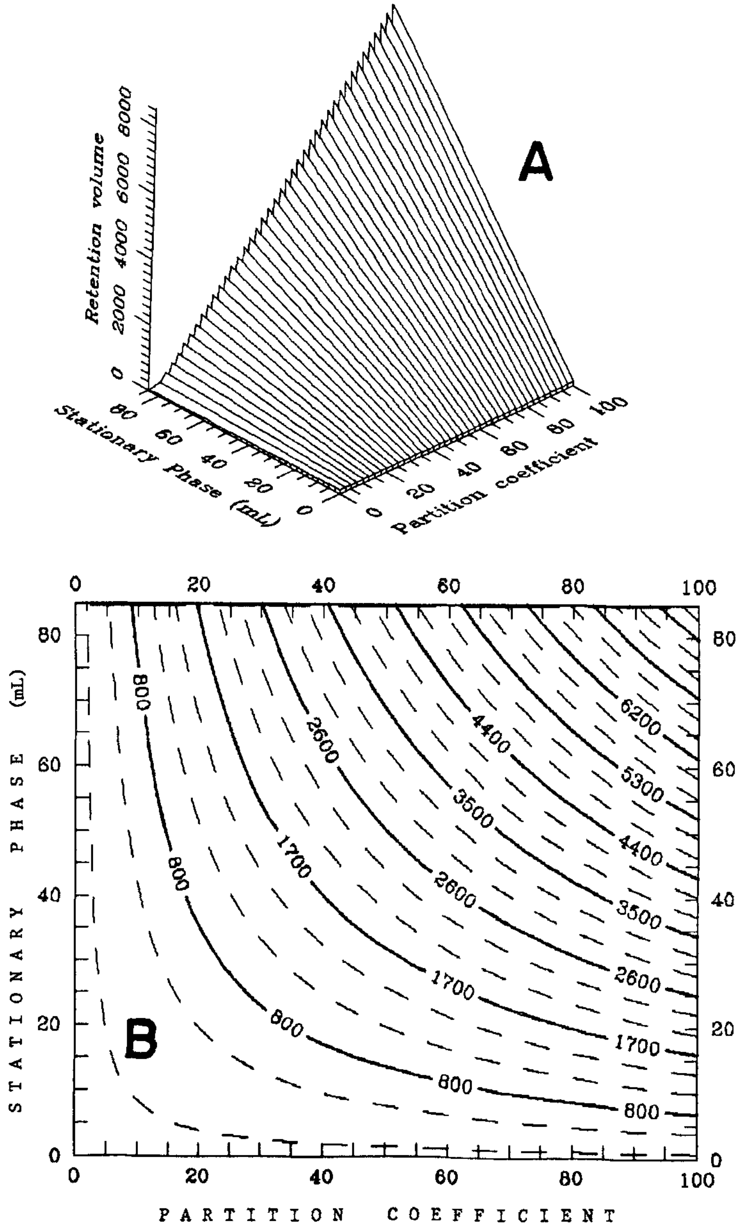

Figure 1: Graphic representation of Equation 2. **A**: 3D-plot with $v_t = 125$ mL, each line correspond to a constant partition coefficient, increment: 2.5. **B**: Isoretention curves (mL) corresponding to the 3D-plot of Figure 1A.

a given compound of partition coefficient K, the retention volume increases linearly with the stationary phase volume. (ii) for an equilibrated system containing a volume, v_s, of stationary phase, the retention volume increases linearly with the partition coefficient of the solutes. Figure 1B presents the isoretention curves corresponding to Figure 1A. From a practical point of view, the maximum flow rate actually usable was about 6 mL/min. A retention volume of 800 mL corresponds to a retention time of 133 min., which is fairly reasonable. But a retention volume of 8000 mL, obtained with 100 or 80 mL as the compound partition coefficient and the stationary phase volume, respectively, produces a retention time longer than 22 hours. The same compound has a retention volume of only 1100 mL, or a retention time of 180 min., when the stationary phase volume is reduced to 10 mL.

If the retention time of a compound is too long, it is possible to change the mode, i.e., to reverse the role of each liquid. The stationary phase becomes the mobile phase and vice versa. In this case, the partition coefficient that should be used in eq. 2 in the reverse mode, corresponds to 1/K, the inverse of the K value in the normal mode. This is sometimes designated K'. For example, the 22 hour retention time compound in the normal mode will have a retention time of only seven min. and 38 s, or a retention volume of 45.8 mL, with 100 for K (0.01 for K'), 80 mL for the stationary phase volume, and 6 mL/min. for the flow rate. Note that this retention volume is only 0.8 mL higher than the dead volume which was 45 mL.

Combining eqs. 1 and 2 and rearranging gives:

$$R_s = [2\,(K_2 - K_1)\,v_s] \,/\, (W_2 + W_1) \qquad [3]$$

This shows that the resolution factor is proportional to the stationary phase volume and to the partition coefficient difference between the two solutes 1 and 2. When two compounds are not well-separated, i.e., the resolution factor (eqs. 1 or 3) is lower than 0.5, it means that the partition coefficients of the two compounds in the liquid system used are very close. To improve the separation, the stationary phase volume can be increased within the physical limits of the system used (cartridge number), or the selectivity can be changed. In CPC, the selectivity adjustment can be done (i) by changing the stationary liquid system, which corresponds to a column change in liquid chromatography; (ii) or by using a ternary liquid system at different compositions, which corresponds to mobile phase changes in liquid chromatography in which a binary miscible liquid system is used as the mobile phase in most analyses. In both cases, the partition coefficients of the two compounds are likely to evolve in different ways producing a better separation. In fact, use of an alkane stationary liquid (such as hexane), along with a mobile phase consisting of water-methanol or water-acetonitrile in CPC gives one a system analogous to reversed phase HPLC with a C_8 or C_{18} stationary phase. Another way to increase the resolution factor is to improve the efficiency, reducing the peak base widths W_1 and W_2.

2-Efficiency

Figure 2 shows a chromatogram obtained with the CPC apparatus. The peak shape is symmetric with very low tailing. In such a case, we can use the classical equation to determine the efficiency, N, of a Gaussian peak:

$$N = 4\,(v_r / W_{0.6})^2 \qquad [4]$$

in which $W_{0.6}$ is the peak width at 60% of the peak height. Since $W_{0.6}$ corresponds to 2 σ, and W, the peak base width, corresponds to 4 σ for a Gaussian peak, one can derive the following expressions from eqs. 3 and 4:

$$R_s = \frac{N^{1/2}\;(K_2 - K_1)}{4(v_o/v_s) + 2(K_2 + K_1)} \qquad [5]$$

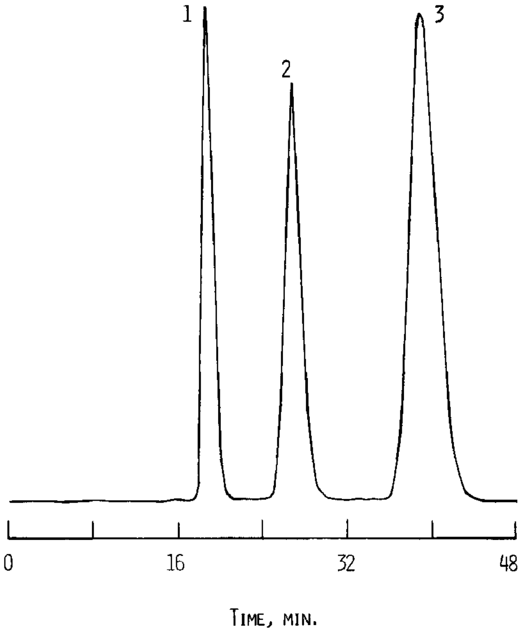

Figure 2: Chromatogram obtained with the system methanol (water 1% v/v)-hexane. Spin rate: 1300 rpm; flow rate (methanol): 3 mL/min., descending mode; wavelength: 230 nm; Absorbance: 0.32; pressure: 35 kg/cm^2; **1**- 1-naphthol (80 μg); **2**- DDD (200 μg); **3**- DDT (400 υg).

Equation 5 shows that, for a given system of immiscible liquids and a given pair of solutes 1 and 2, the resolution factor is maximum for the highest value of v_S, that minimizes the ratio v_0/v_S. The resolution factor is also proportional to the square root of the plate number, N, as it is in classical liquid chromatography.

To study the efficiency of a chromatographic system, a van Deemter plot is drawn by plotting the plate height versus the flow rate (6). The plate height or height equivalent to a theoretical plate (HETP) is the ratio of the

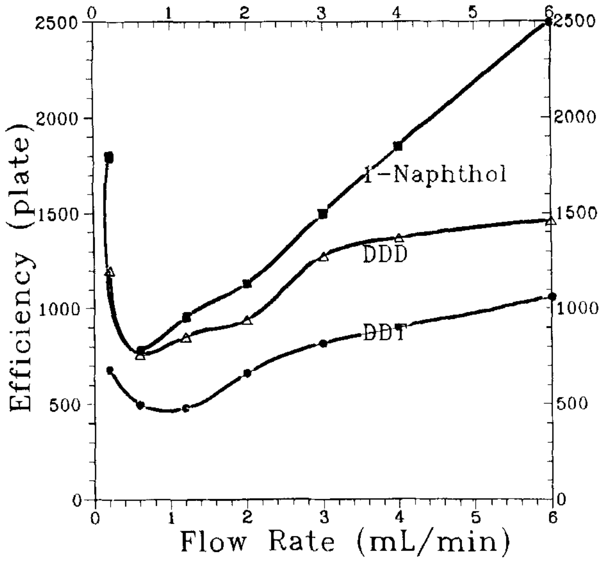

Figure 3: Efficiency versus flow rate for the system methanol (waver 1% v/v)-hexane in the descending mode (stationary phase: hexane). The solutes are the same as those in Figure 2. The runs were made at 20°C and 1100 rpm.

column length to the plate count, N. In CPC, there is not actually a column. In order, to make the CPC "van Deemter" plot, one must plot the ratio (2400/N) versus the flow rate. As there are 2400 channels in the apparatus of this study, the maximum plate count is 2400. The ratio 2400/N represents the number of channels needed to produce one theoretical plate.

Figure 3 shows the effect of flow rate on efficiency obtained for the three compounds in Figure 2 and using a mobile phase of methanol, water 1%, v/v)-hexane at 25°C. At very low flow rates, the efficiency decreases with increasing flow rates up to about 0.7 mL/min. Above 0.8 mL/min., the opposite trend is observed: the efficiency increases when the flow rate increases. Figure 4 shows the data of Figure 3 graphed in the same manner as

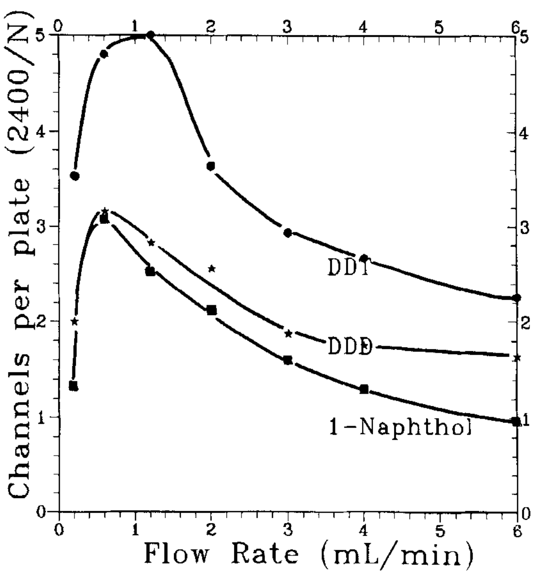

Figure 4: A van Deemter-type plot where the number of channels required to obtain one theoretical plate is plotted versus the flow rate. The solutes are the same as those in Figure 2. The runs were made at 20°C and 1100 rpm.

most traditional van Deemter plots for gas and liquid chromatoraphy (5). Usually in liquid or gas chromatography, there is an optimum flow rate. Below or above this optimum, the efficiency decreases or the HETP increases. The *opposite* is observed in CPC, there is a distinct flow rate with the worst efficiency (Figure 3) or the highest channel number per theoretical plate (Figure 4).

This type of van Deemter plot shape is not specific for the methanol//water-hexane solvent system. The same general trend was obtained with the octanol-water system and two other solutes (pentachlorophenol and hydroquinone). Table I lists the results obtained with both liquid systems and five solutes.

TABLE I - Efficiency minima for different systems[a]

Liquid system	Solute	K	flow mL/min.	Re	Channels per plate	N
Methanol (1% v/v water) Hexane	1-naphthol	0.17	0.6	4.8	3.1	775
			6.0	47.7	1.0	2400
	DDD	0.47	0.7	5.6	3.2	750
			6.0	47.7	1.7	1420
	DDT	0.96	1.0	8.0	5.1	470
			6.0	47.7	2.3	1040
Octanol-water	pentachlorophenol	10^{-5}	1.8	1.9	4.4	550
			6.0	6.3	2.8	850
	hydroquinone	0.34	3.0	3.2	18.0	130
			6.0	6.3	13.4	180

[a] The first liquid was the mobile phase, K is the partition coefficient used in equation 2, Re is the Reynolds number (see text). The efficiency at 6 mL/min. is given with the minimum efficiency for comparison. N is the number of theoretical plates in that experiment.

For each system, the efficiency increases toward 2400 as the flow rate approaches zero. For all other flow rates, the efficiency seems to be liquid system dependent. It is much lower with the octanol-water system. The viscosity of octanol very likely decreases the mass transfer between phases. The efficiency seems also to be solute dependent: the higher the solute retention, the lower the efficiency. The flow rate, corresponding to the minimum of efficiency, seems also to be liquid system and solute dependent.

To interpret the unusual "van Deemter" plot obtained in CPC, the following theory is proposed. At low flow rates, a laminar liquid stream exists in the channels. The mobile phase goes through the stationary phase in the form of monodisperse droplets. When the flow rate increases, the droplet number increases, but the residence time of a given droplet in the stationary phase is the same (7). When the flow rate is reduced, the droplet number decreases. The mass transfer depends on the interfacial area, the diffusion coefficient of the solute and on the kinetics of the exchange between the stationary phase and mobile-phase. The interfacial area corresponds to the droplet's surface plus the liquid interface at the top and the bottom of each channel. When the flow rate becomes very low, the mass transfer occurring at this interface becomes more important than the droplet mass transfer. At the limit, when the flow rate is nil, there is no droplet, the mass transfer takes place only at the liquid interface at the top and bottom of each channel, and there is an infinite equilibration time producing the maximal efficiency: 1 channel = 1 theoretical plate. Since the efficiency decreases when the flow rate increases, the droplet traveling time through the stationary phase is not long enough to obtain a complete equilibrium of the solute in the phases. Several channels are necessary to obtain one theoretical plate.

In each of the cases studied, the efficiency versus flow rate presents a minimum. One possibility is that this minimum corresponds to a change in the hydrodynamic state of the liquid mobile phase. In order to find out whether or not this is true it is necessary to calculate Reynolds numbers for our system. These numbers are used to evaluate the hydrodynamic state of flow streams. For a lineary pipe, the Reynolds number is defined as:

$$Re = (d\, v\, \rho) / \mu \qquad [6]$$

in which d is the pipe diameter (cm), v and ρ are the fluid velocity (cm/s) and density (cm^3/s), respectively, and μ is the fluid viscosity (cP or g/(cm x s x 100)) . The fluid velocity depends on the flow rate :

$$v = (4F) / (\pi d^2) \qquad [7]$$

Using the flow rate instead of the velocity, we get:

$$Re = (4\, F\, \rho) / (\pi\, d\, \mu) \qquad [8]$$

with the flow rate, F, in mL/s. Table I presents the Reynolds numbers corresponding to the minimum efficiency flow rate and to 6 mL/min. These numbers were calculated using 3.4 mm as the channel equivalent pipe diameter (see Table I in Reference 1). This value was obtained from pressure increase studies by calculating the diameter of the open tube of length, h, the channel height, equivalent to one channel (1). For a linear pipe, the transition from a laminar flow to a turbulent flow occurs for a Re value of 2000. The Re values obtained in our study (Table I) lie between 1.9 and 48, i.e., well below 2000. The 2000 value is valid for a linear open tube of constant diameter. In our study the direction of flow changes by 180° every few centimeters and the flow channel width changes with every change in flow direction (see Figure 2-4 in Ref. 1). In a previous study of the acetic acid exchange between toluene droplets and water, Streicher and Schugerl found a flow change for a Reynolds number of 35 in irregular channels (7,8). Given the crooked shape and different diameters of the CPC channel and its duct (1), it may be possible that there is a transition from classical laminar to some type of nonlaminar flow, at least locally, for a Reynolds number as low as 2 (see Table I). If there is a mixed-mode flow or the droplets collide with the wall of the channel, or an emulsified layer is formed, then the droplet size would no longer be constant and the interfacial area between the stationary and mobile phases would increase. These factors plus better mixing would make the mass transfer more efficient. As the efficiency increases, the number of channels needed to correspond to one theoretical plate decreases (Figures 3 and 4).

Table I and Figures 3 and 4 show that the efficiency minimum is somewhat solute dependent, that is solutes with higher P values, have slightly higher flow rates which correspond to their minimum of efficiency. Obviously, the flow rate at which classical laminar flow ceases to be dominant cannot be solute-dependent. With the methanol-hexane system, this change seems to occur at a flow rate of 0.6 mL/min. (Re = 4.8). Figure 4 shows that at this value the curvature of the efficiency of the three solute studied changes. The same observation holds true with octanol-water system in which the flow transition begins at 1.8 mL/min. (Re = 1.9).

The study of other liquid systems and solutes is in progress in our laboratory. With those data, it might be possible to derive a general equation representing the "inverse van Deemter" plot obtained with CPC. From a practical point of view, the fact that the efficiency increases at high flow rate is very favorable. The best separations or partition coefficient determinations are obtained in the shortest time. Unfortunately it is not possible to increase continuously the flow rate without eventually encountering restrictions. The two factors limiting the flow rate are: (i) the "flooding" point and (ii) the pressure limitation. The "flooding" point corresponds to a definite flow rate at which emulsion formation occurs. The stationary phase is pushed out of the rotor in the form of tiny droplets. To avoid this problem, it is possible to hold the stationary phase more tightly in the rotor by increasing the spin rate. Then the pressure becomes a limiting factor. We have shown that the pressure can be represented by (1):

$$P = n\,[\Delta\rho\, w^2\, R\, h + \eta\,\gamma\, F] \qquad [9]$$

in which n is the total number of channels (2400), $\Delta\rho$ is the density difference between the mobile and the stationary phase (g/cm^3), w is the spin rate (rd/s), R is the rotor radius (cm), h is the channel height (cm), η is the mobile phase viscosity (cP), γ is a geometric characteristic of the channel and duct system (cm^{-3}), and F is the flow rate (mL/min.). The pressure increases linearly with the flow rate, and quadratically with the spin rate. Because the channels are engraved in a PCTFE plate and the cartridges are interconnected by teflon tubing, the apparatus, and especially the rotary seals cannot withstand a pressure higher than 60 kg/cm^2 (or 850 p.s.i.). With the liquid systems of this study, the optimal spin rate was 1100 rpm and 800 rpm for the system methanol-hexane and octanol-water, respectively; in both cases the "flooding" point was around 7 mL/min.

ABBREVIATIONS AND SYMBOLS

CCC:	Counter Current Chromatography
CPC:	Centrifugal Partition Chromatography
cP:	Centipoise
3D:	Three Dimensional
DDD:	1,1-p-Chlorophenyl-2,2-dichloroethane
DDT:	2,2-p-Chlorophenyl-1,1-trichloroethane
d:	Tube Diameter (cm)
PCTFE:	Polychlorotrifluoroethylene
F:	Flow Rate (mL/sec)
h:	Height of the Stationary Phase in the Channel (m)
hc:	Channel Height (m)

K:	Partition Coefficient
K':	1/K
L:	Tube Length
n:	Number of Channels
N:	Plate Number
P:	Pressure (Newtons/m^2)
p.s.i.:	Pounds Per Square Inch
r:	Tube Radius
R:	Rotor Radius
Re:	Reynolds Number
R_s:	Resolution
t:	Retention Time
v_o:	Dead Volume
v_r:	Retention Volume
v_s:	Stationary Phase Volume
v_t:	Total Internal Volume
w:	Rotor Spin Rate (Radians)
W:	Baseline Peak Width
$W_{0.6}$:	Peak Width at 60%, Peak Height (i.e., 2 σ)
η:	Liquid Viscosity (kg/ms)
γ:	$8L/\pi r^4$
ν:	Fluid Velocity (cm/s)
π:	3.1416
ρ:	Liquid Density (kg/m3)
$\Delta\rho$:	Density Difference Between Mobile and Stationary Phases
σ:	Standard Deviation

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