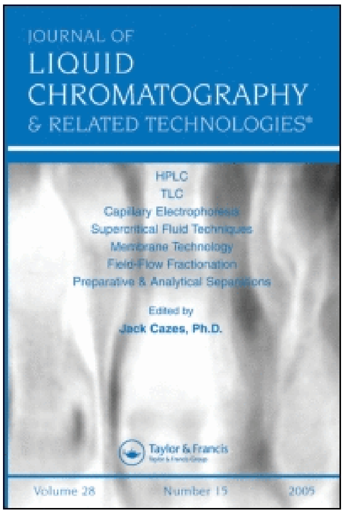

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Centrifugal Partition Chromatography. I. General Features

Alain Berthod[a]; Daniel W. Armstrong[ab]
[a] Laboratoire des Sciences Analytiques, Universite de Lyon-1, Villeurbanne, France [b] Department of Chemistry, University of Missouri-Rolla, Rolla, Missouri

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CENTRIFUGAL PARTITION CHROMATOGRAPHY. I. GENERAL FEATURES

Alain Berthod[1], and Daniel W. Armstrong*
Department of Chemistry
University of Missouri-Rolla
Rolla, Missouri 65401

ABSTRACT

Centrifugal Partition Chromatography (CPC) is a variant of countercurrent chromatography (CCC). 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 that a mobile phase can be pumped through it. This system is analyzed in detail. The stationary phase evolution versus time is studied. A complete derivation is made of the relationship linking system pressure to the spin and flow rate as well as to the physico-chemical properties of the two liquids, i.e., density and viscosity.

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[1] On leave from Laboratoire des Sciences Analytiques, Universite de Lyon-1, U.A.CNRS 435, 69622 Villeurbanne, France

INTRODUCTION

For years, the differential partitioning of compounds between two immiscible liquids has been used for extraction and purification purposes (1). Ito introduced countercurrent chromatography (CCC) as an improvement on stepwise countercurrent extraction (2). CCC utilized two immiscible liquids, one as the stationary phase, the other as the mobile phase. The two immiscible liquids could be moved countercurrently in a helical rotating tube. Rotation creates an acceleration field that keeps one of the liquids stationary while the other liquid is mobile. Reviews on CCC have been published recently (3-6).

Some advantages of CCC are (i) the ability to separate molecules of a broad range of molecular weights, from drugs, pesticides and natural products to blood particles and cells. (ii) As there is no solid support, if a compound is strongly retained by the stationary phase, it can be easily recovered by flushing the system. (iii) Given the possible large volume of stationary phase, CCC is well suited for preparative separations or purifications (7).

Ito and others developed CCC introducing locular CCC (8) and droplet CCC (9). A droplet CCC chromatograph is formed by a set of vertical columns interconnected in series by capillary tubes. The system is filled with the stationary phase, then the mobile phase is pushed through the stationary phase forming a stream of droplets surrounded by stationary phase. Centrifugal partition chromatography (CPC) is very close to droplet CCC. Basically, to improve the efficiency, the vertical columns were replaced with channels machined into a polychlorotrifluoroethylene (PCTFE) plate. To reduce the analysis time, those plates were rotated in a centrifuge to increase the field (10,11).

The aim of this paper is to present the CPC technique and its capabilities. An equation is derived relating the system back pressure to all relevant parameters. The physical characteristics of CPC are compared with analytical and preparative chromatography with a solid stationary phase. Because the basic retention equation uses the stationary phase volume, special attention is given as to the determination and control of the stationary phase volume.

EXPERIMENTAL SECTION

1-Apparatus

The CPC experiments were performed using a Sanki centrifugal partition chromatography, Model CPC-NMF, from Sanki Laboratories Inc., Sharon Hill, PA. This apparatus consists of up to 12 cartridges placed in the rotor of a centrifuge. Figure 1 is a picture of a rotor containing only six cartridges. Each cartridge is composed of four PCTFE plates separated by five teflon sheets. Each PCTFE plate bears 100 channels engraved in two rows of 25 on each side.

Figure 2 shows the channel design. For clarity, only 14 channels, instead of 50, have been drawn. In each row, the top of the channel is connected to the bottom of the next one. The top of the last channel of a row is connected to the bottom of the first channel of the next row, either through a duct on the same side of a PCTFE plate (bottom of Figure 2) or through a hole across the plate to the other side. The liquid must travel through the 100 channels of a PCTFE plate , then it goes to the next plate, through the 400 channels of a cartridge, then it goes to the next cartridge and so on, as shown by Figure 1. During operation at 800 rpm the internal volume of a cartridge is approximately 20 mL corresponding to about 50 μL per channel and

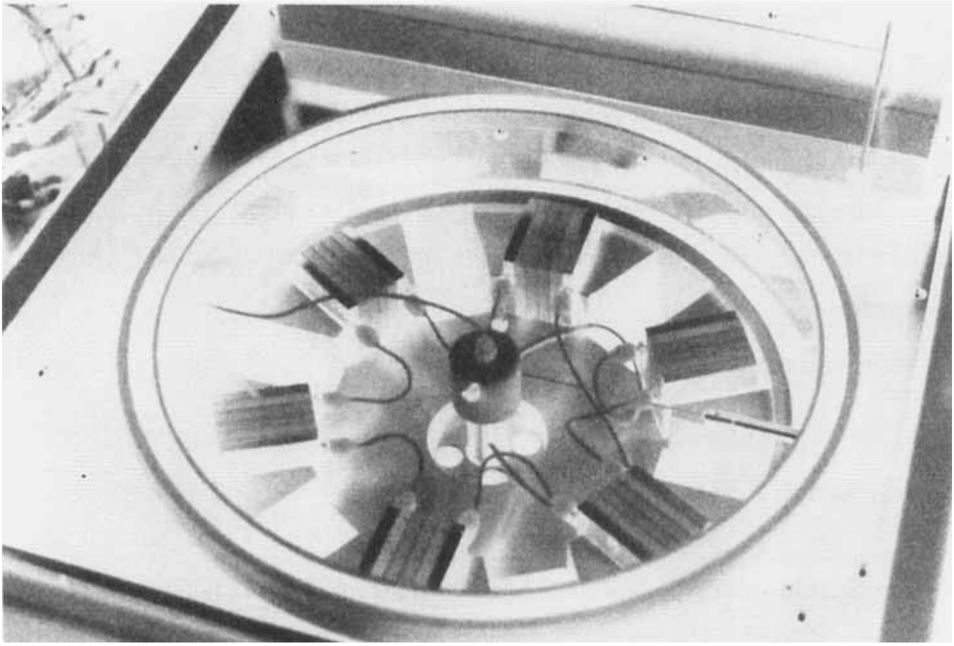

Figure 1: The rotor of the CPC apparatus loaded with six cartridges. Up to 12 cartridges can be loaded. Note the connecting tubing and the upper rotary seal.

connecting duct (see Figure 2). When the centrifuge rotor is loaded with six cartridges, the internal volume from the injection valve to the detector output is 125 mL.

The general setup of the apparatus is shown in Figure 3. The rotor is enclosed in a constant temperature box, all experiments were done at 25°C. A classical liquid chromatograph pump (Shimadzu, Model LC 6A or Waters, Model 590) was used. The Sanki command module (Model CPC-FCU-V) allowed one to inject a sample (valve V1 on Figure 3) and to choose between the ascending or descending mode (valve V2, Figure 3). Those two modes

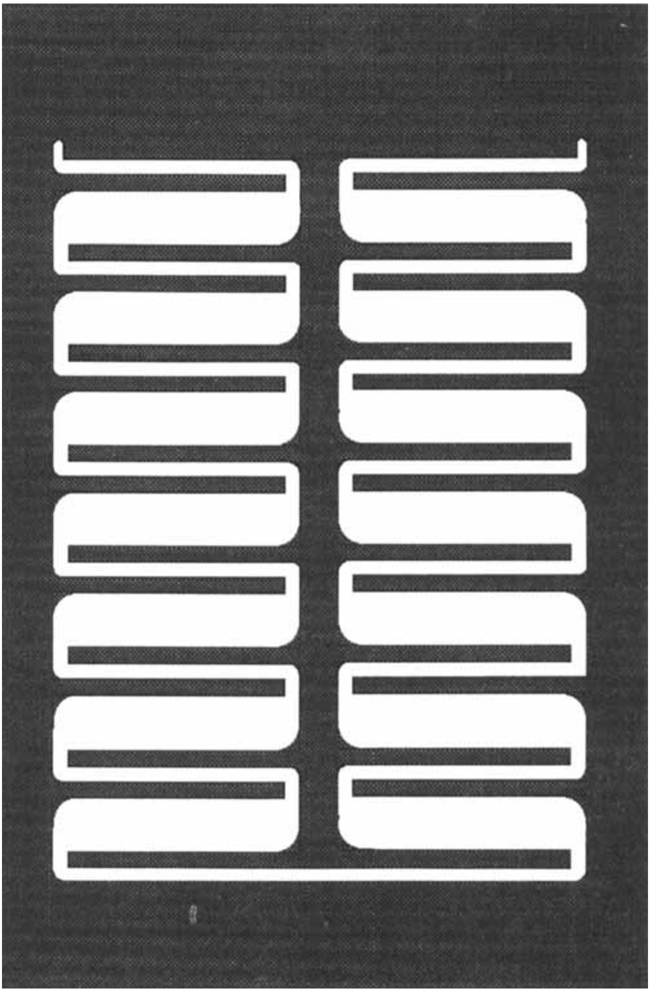

Figure 2: The channel design. An actual cartridge contains 400 channels in four PCTFE plates, with 25 channels on each side of each PCTFE plate. Only 14 channels are represented here for clarity. At rest, each channel and connecting duct is 12.4 mm long and 1.1 mm deep. The width of the channel was 2.4 mm while that of the duct was 0.9 mm. The dividing wall between the channel and connecting duct was 1.1 mm wide. The rounded corners of ducts and channels have radii of curvature of approximately 0.45 mm and 1.0 mm respectively.

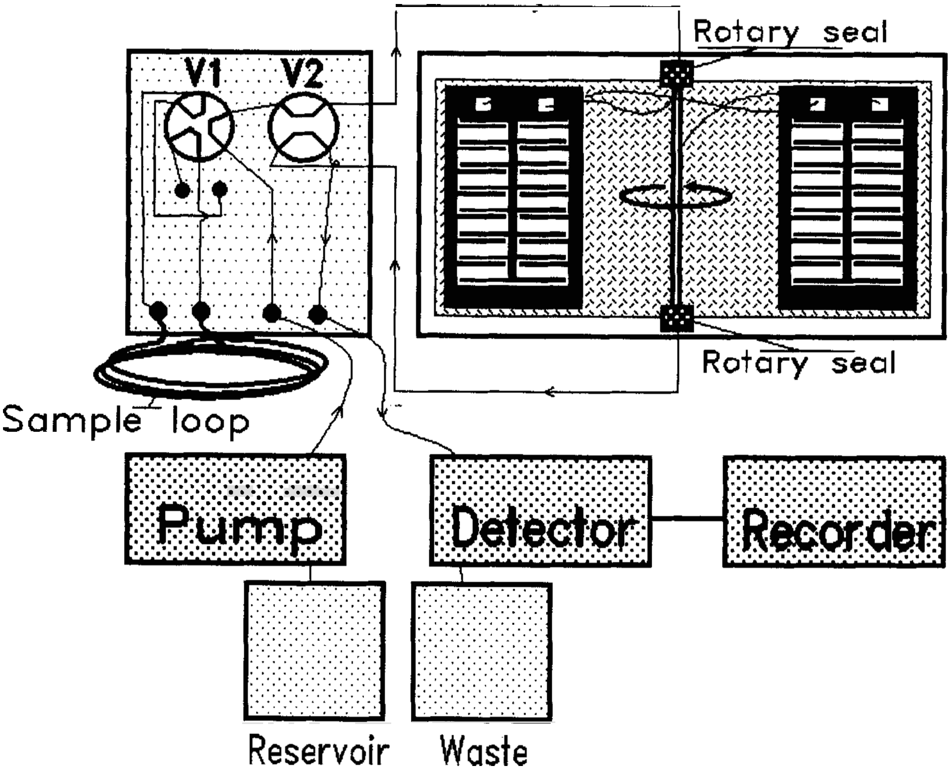

Figure 3: The general setup in the descending mode. V1 = injection valve; V2 = switching valve (ascending or descending mode). The sample loop volume is 3 mL.

will be fully described latter. The detector was a Shimadzu, Model SPD-6A, UV-Visible detector equipped with a preparative flow cell. The recorder was from Linear Inc., Model 1200.

2-Methods

A system of two immiscible liquids is chosen. In this study, the systems water-octanol and methanol (1% v/v water)-hexane were used. The two immiscible liquids are mixed together and allowed to equilibrate in an ultrasonic bath for at least half an hour, in order to be sure that each phase is well saturated with the other one, Then, according to several factors that will be discussed below, the ascending or descending mode is chosen. In the descending mode, the stationary phase is the upper and lighter liquid. The apparatus is first filled up with the stationary phase. Only then is the denser mobile phase pumped through the stationary phase from the top of the centrifuge to the bottom. The stationary phase is maintained in each channel by the centrifugal field created by the rotor spin. In Figure 3, the valve V2 is positioned in the descending mode as indicated by the arrow on the connecting tubes. Figure 4 shows a simplified view of the liquid behavior in the channels in the descending mode. The mobile phase passes through the stationary phase in the form of tiny droplets moving in the centrifugal field, G. In the ascending mode, the stationary phase is the lower and denser liquid, and the mobile phase is the less dense liquid. The mobile phase moves from the bottom to the top of the apparatus. The droplets of mobile phase move through the stationary phase against the centrifugal field.

RESULTS AND DISCUSSION

1-Basic Retention Equation

The retention of any compound in CPC obeys the classical chromatographic equation:

$$v_r = v_o + K\, v_s \qquad [1]$$

in which v_r, v_o and v_s are the retention volume, the dead volume and the stationary phase volume, respectively. K is the partition coefficient of the compound between the mobile and the stationary phase.

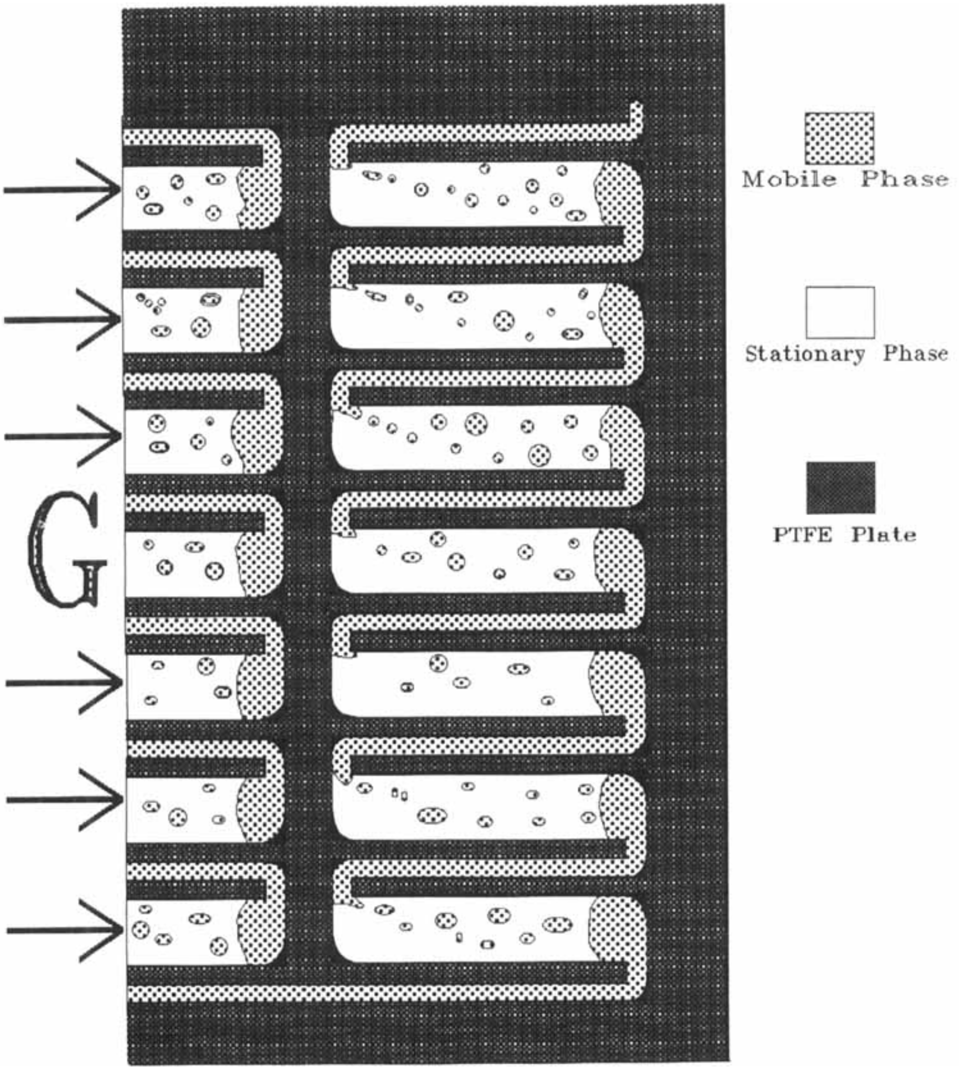

Figure 4: An oversimplified representation of the mobile phase motion through the stationary phase in the descending mode. The mobile phase is more dense than the stationary phase. G is the centrifugal field induced by the rotor spinning.

The stationary phase volume and the dead volume are not independent. Their sum correspond to the total internal volume of the apparatus, v_t:

$$v_o + v_s = v_t \qquad [2]$$

Combining eq. 1 and 2, we get the convenient equation:

$$v_r = v_t + (K - 1)\, v_s \qquad [3]$$

As v_t is constant, if the stationary phase volume, v_s, is known, the measurement of the retention volume, v_r, allows one to calculate the partition coefficient P of a compound though eq. 3. On the other hand, the retention volume of a compound whose partition coefficient is known leads to the stationary phase volume.

2-Decrease of the Stationary Phase Volume

Column "bleeding" is a well-known problem with coated columns in gas or liquid chromatography. A compound is coated on the solid support and used as the stationary phase. This compound is slowly eluted by the mobile phase, producing baseline drifts and shifts of the retention times. This problem also exists in CPC: the stationary phase volume slowly decreases as the mobile phase is pumped through. Figure 5 shows the evolution of the stationary phase (water) volume versus time when octanol is the mobile phase. To obtain those results, the solute pentachlorophenol was used. This solute is very soluble in octanol, with 105,000 as the partition coefficient versus water. When octanol is the mobile phase, the K value to be used in eqs. 1 or 3 is $1/105{,}000 = 9.5 \times 10^{-6}$. Equation 1 shows that the product Kv_s is the difference $v_r - v_o$. With 80 mL as the maximum value for

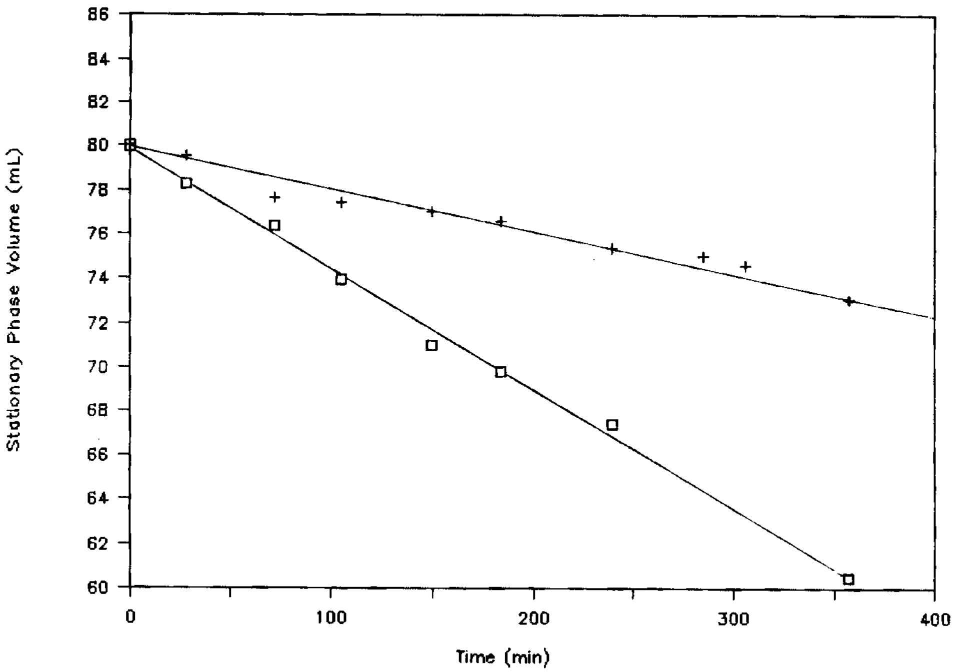

Figure 5: Evolution of the stationary phase volume versus time. Mobile phase: octanol, stationary phase: water (pH 2), six cartridges, spin rate: 800 rpm, flow rate: 2 mL/min, test solute: pentachlorophenol. Cross: well-saturated mobile phase, Open square: unsaturated mobile phase.

the stationary phase volume (Figure 5), the Kv_S product is only 0.76 μL. This retention volume can be added to the dead volume and pentachlorophenol can be used as a dead volume tracer.

The squares of Figure 5 corresponds to an octanol mobile phase saturated with water after shaking and standing 15 min. in an ultrasonic bath. The crosses correspond to an octanol mobile phase saturated with water after one hour in the ultrasonic bath and a manual shaking of ten minutes. The dissolution of the stationary phase by the non-saturated octanol is obvious. However, even with a well-saturated mobile phase some dissolution may occur. This is because the liquids are allowed to equilibrate at atmospheric pressure, then, inside the apparatus, at high pressure (up to 50 kg/cm^2), the mutual solubility of the phases may increase. Also, the stationary phase may be pushed out in the form of microscopic droplets, although the mobile phase exiting the detector seems to be clear.

The stationary loss was 3.3 mL per hour (4%) and 1.05 mL per hour (1.3%) for the squares and the crosses on Figure 5, respectively. That corresponded to 27.5 mL and 8.5 mL of water per liter of octanol, respectively. At atmospheric pressure and 25^{o}C, the water concentration in octanol at saturation is 41.4 mL per liter. This constant "column bleeding" must be kept in mind and, in the accurate determination of partition coefficients, dead volume tracers and a correction for stationary phase variation must be used. It also should be noted that this bleeding is very dependent on the properties of the chosen solvents, flow rate and system pressure.

3-Preparing the CPC System

In LC, each time a new mobile phase has to be used with a given column, or after a gradient run, the column (stationary phase) must be equilibrated with the new mobile phase. Something similar must be done with the CPC system. When a new liquid system has to be used, the first step is to fill up the cartridges with the stationary phase liquid at a low spin rate. Once the system is filled with the stationary phase, the pump and valves are flushed with the mobile phase. The mode is chosen according to the respective density of each phase, the spin rate is increased to the working value, and the mobile phase is pumped through the stationary phase. A graduated cylinder replaced the waste tank to measure the volume of stationary phase pushed out by the mobile phase.

Figure 6 shows the pressure evolution when the system is equilibrated with the mobile phase. The trend is always the same, whatever the liquid system or the mode, ascending or descending: the pressure increases linearly as the mobile phase displaces the stationary phase from the connecting ducts (Figures 2 and 4). The pressure sensor of the Waters pump (Model 590) was not sensitive to pressure lower than 10kg/cm^2, which is why curves 1 and 3 (Fig. 6) do not cross the origin as does curve 2, obtained with the Shimadzu pump. When the first drop of mobile phase comes out, the pressure stabilizes (arrow on Figure 6) and starts to decrease slowly as described in the previous paragraph (section 2). It can be seen that the observed pressure is proportional to the amount of stationary phase through which the mobile phase passes. The slopes of the linear parts of curves 1, 2 and 3 are 1.21; 0.678; and 0.348 kg/(cm^2 mL), respectively.

4-Parameters Acting on the Pressure Drop

If we consider a channel filled with a liquid in a gravitational field G, the pressure difference ΔP (in newtons per square meter, N/m^2) between the bottom and the top of the channel is given by the classical law of hydrostatics:

$$\Delta P = \rho\, G\, h_c \qquad [4]$$

in which ρ is the liquid density (kg/m^3), and h_c is the channel height (m).

When only one liquid is in the system, all channels are connected by ducts (Figure 2). Then, in each duct, the pressure drop due to the hydrostatics compensates that in the channel (siphon effect). When a second liquid is pushed into the system, the pressure drop in the duct (which contains

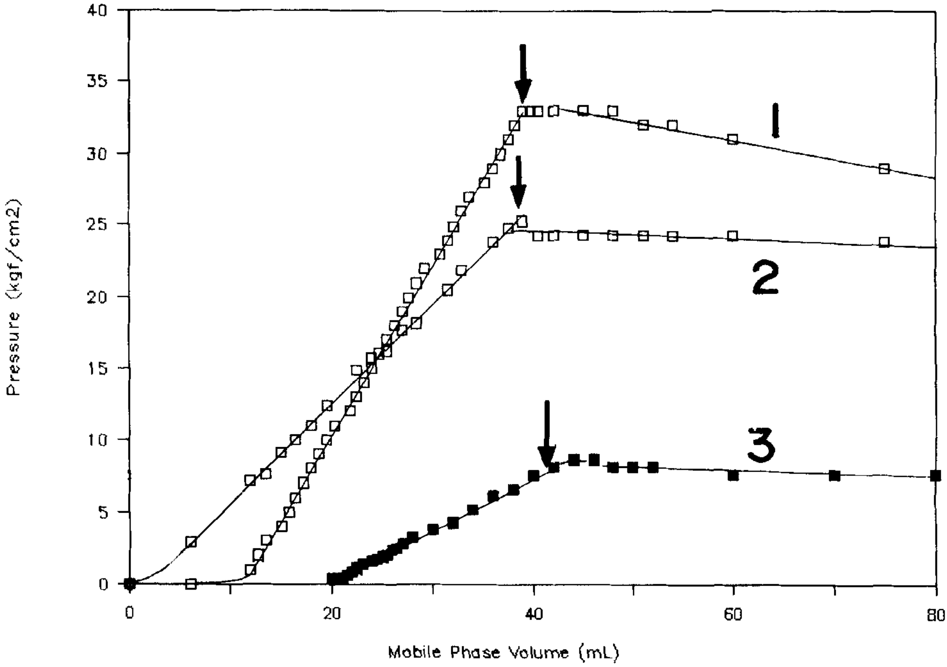

Figure 6: Pressure drop during the equilibration of the system. Open square: octanol/water, 1- octanol mobile phase, 800 rpm; flow rate 3 mL/min; ascending mode (pump Waters). 2- Water mobile phase, 700 rpm; 3 mL/min; (pump Shimadzu). Close square: methanol (1% water)/hexane 3- methanol mobile phase, 800 rpm, 2 mL/min, descending mode. (pump Waters). The arrows indicate the first drop of mobile phase exiting the apparatus.

only the second liquid) does not compensate exactly the pressure drop in the channel (which contains both liquids). The residual pressure depends on the density difference, $\Delta\rho$, between the two liquids and the height, h, of the stationary phase in one channel:

$$\Delta P = \Delta\rho \, G \, h \qquad [5]$$

This phenomenon occurring for each channel, and the centrifugal field being expressed as $w^2 R$, in the case of a rotor of radius, R, the hydrostatic pressure in the system can be expressed as :

$$\Delta P = n\, \Delta\rho\, w^2\, R\, h \qquad [6]$$

in which n is the total number of channels; w is the spin rate (radians); R is the rotor radius (m) and h is the stationary phase height in a channel (m).

In addition to the hydrostatics term, represented by eq. 6, a hydrodynamic term must be present in the pressure drop equation. This term can be derived from Poiseuille's law for an open tube:

$$\Delta P = 8\ \eta\, L\, F/(\pi\, r^4) \qquad [7]$$

in which ΔP is the driving pressure (N/m^2), η is the liquid mobile phase viscosity (kg/(m x s) or Poise x 0.1 or cP x 0.001), L and r are the tube length and radius, respectively (m); and F is the flow rate (cm^3/s). The ratio $8\ L / \pi\ r^4$ corresponds to geometrical characteristics of the tube, it will be referred to as the coefficient γ (m^{-3}).

At the beginning of an experiment, when the mobile phase is pushed through the stationary phase, the pressure increases linearly (Figure 6). The mobile phase fills up the ducts of a linearly increasing number of channels. Given the results presented by Figure 6, and assuming that the stationary phase height, h, is equal to the channel height, h_c, it is possible to add the hydrostatic term (eq. 6) and the hydrodynamic term (eq. 7) to get:

$$P = n\, [\, \Delta\rho\, w^2\, R\, h\ + \eta\, \gamma\ F] \qquad [8]$$

Even though the crooked shape of the channels (Figure 2) is certainly not an open tube of constant radius, the γ value of eq. 8 corresponds to an open tube of geometrical characteristics equivalent to a channel and its duct. Equation 8 links the experimental driving pressure to the physico-chemical properties of the liquids used and to the spin and flow rate. Of these factors, the linear increase of the driving pressure with the density difference between the two liquid phases was reported by Murayama et al. (11).

The relative importance of the hydrostatic and hydrodynamic terms of eq. 8 depends greatly on the experimental conditions. If the density difference between the two immiscible liquids is high and the viscosity is low (system hexane-water, for example), the hydrostatic term will be the principal factor acting on the pressure drop. The flow rate will have a slight effect. For example, with the system hexane water ($\Delta\rho$ = 0.339 g/cm^3 and η_{hexane} = 0.29 cP), with 3 mL/min and 500 rpm as the flow and spin rates, respectively, the hydrodynamic term contributes to the total pressure drop for only 3.6%. On the other hand, when the viscosity of the mobile phase is high (octanol, for example) and the density difference is low, the hydrodynamic term of eq. 8 becomes more important. With the system octanol-water ($\Delta\rho$ = 0.137 g/cm^3 and $\eta_{octanol}$ = 5 cP), 3 mL/min. and 500 rpm, the hydrodynamic term corresponds to 61% of the total pressure drop.

The spin rate remains the most important parameter. It increases the pressure drop quadratically. For the octanol-water system, a 60% spin rate increase (from 500 rpm to 800 rpm) multiplies by 2.5 the pressure drop due to the hydrostatic term. In such conditions (octanol-water system, 3 mL/min and 800 rpm), the hydrodynamic term of eq. 8 contributes only 38% to the total pressure drop.

Table I presents the physico-chemical characteristics of some liquid systems used in this study. The results obtained preparing six "columns" are

Table I. Physico-Chemical Parameters and Pressure Increase for Some Liquid Systems

#	Stationary phase	Mobile phase	Δρ g/cm^3	w radians/s	η cP	F cm^3/s	slope $(kg/cm^2)/mL$
1	Water	Octanol	0.137	83.8	5.0	0.050	1.210
2	Octanol	Water	0.137	73.8	1.12	0.050	0.678
3	Hexane	Methanol (water 1% v/v)	0.061	83.8	0.57	0.033	0.348
4	Hexane	Methanol (water 10% v/v)	0.143	83.8	0.80	0.033	0.810
5	Methanol (water 1% v/v)	Hexane	0.061	83.8	0.31	0.033	0.335
6	Hexane	Methanol (water 1% v/v)	0.061	136.1	0.57	0.033	0.955

PRESSURE DROP

#	Stationary phase	Mobile phase	Δρ/cm or g/cm^5	Δρ/channel or g/cm^5	γ cm^{-3}	r mm
1	Water	Octanol	1210	20.17	3280	1.7
2	Octanol	Water	678	11.3	3840	1.6
3	Hexane	Methanol (water 1% v/v)	348	5.8	2530	1.8
4	Hexane	Methanol (water 10% v/v)	810	13.5	3490	1.7
5	Methanol (water 1% v/v)	Hexane	335	5.3	2540	1.8
6	Hexane	Methanol (water 1% v/v)	955	15.9	9850	1.3

Density difference and viscosity are for the mobile phase saturated by the stationary phase.

presented, preparing a column being taken in the sense of equilibrating the cartridges with a new liquid system. The three first experiments are represented graphically by Figure 6.

The slopes of Table I correspond to the pressure increase produced by 1 mL of mobile phase (Figure 6). Noticing that the mobile phase volume is about 40 mL (+/- 1 mL) with the 2400 channel system, whatever the liquid system used (arrows on Figure 6) , it can be estimated that 1 mL of mobile phase fills up 60 channels (2400/40), so the increase of pressure due to the addition of one channel corresponds to the slope of the straight lines (Figure 6) divided by 60. Then, the γ value of eq. 8 can be estimated with:

$$\gamma = [\Delta P \text{ per channel} - \Delta\rho\, w^2\, R\, h] / \eta\, F \qquad [9]$$

Using the estimated h value of 10.5 mm (10) as the length of the channel equivalent open tube, its radius is given by:

$$r = (8\, h / \pi\, \gamma)^{0.25} \qquad [10]$$

The radius of the channel equivalent tube is shown in Table I. A remarkable constancy of the r value must be noted. The mean value is 1.7 mm +/- 0.1 mm. We discard the last value (1.3 mm) because it was obtained in an experiment at high spin rate (1300 rpm) for which we suspect a dissolution of the hexane stationary phase by the methanol mobile phase. This dissolution may be due to the high pressure (30 kg/cm^2) inside the cartridges and to some heat generated by the rotary seals (see part III of this series (12)). The constancy of the r and γ values (Table I) speaks for the validity of eq. 8.

When the stationary phase is dissolved or pushed out by the mobile phase, the height, h, decreases, then as predicted by eq. 8, the pressure decreases at constant spin and flow rate. Indeed, by watching the pressure during several runs, it is possible to know if the stationary phase is dissolved or pushed out.

To conclude this paper, we would like to emphasize that it is possible to use the CPC apparatus as a classical chromatograph. Once the cartridge system is loaded with the stationary phase and equilibrated with the mobile phase, it can be used as a classical column. Watching the pressure allows one to take into account any column "bleeding". At any time, it is possible to load some stationary phase volume, by injecting the solutes dissolved in the stationary phase, for example. This produces a pressure increase, the flow and spin rate being constant. In the following paper of this series, the selectivity and the mass transfer between both phases will be evaluated (13).

ABBREVIATIONS AND SYMBOLS

CCC:	Counter Current Chromatography
CPC:	Centrifugal Partition Chromatography
PCTFE:	Polychlorotrifluoroethylene
F:	Flow Rate (cm^3/s)
G:	Gravitational Constant
h:	Height of the Stationary Phase in the Channel (m)
h_c:	Channel Height (m)
K:	Partition Coefficient
L:	Tube Length
n:	Number of Channels
N:	Newton
P:	Pressure (N/m^2)
ΔP:	Pressure Difference (N/m^2)
r:	Channel Equivalent Tube Radius
R:	Rotor Radius
v_o:	Dead Volume
v_r:	Retention Volume

v_s: Stationary Phase Volume
v_t: Total Internal Volume
w: Rotor Spin Rate (Radians)
η: Liquid Viscosity (kg/ms)
γ: $8L/\pi r^4$
π: 3.1416
ρ: Liquid Density (kg/m^3)
$\Delta\rho$: Density Difference

ACKNOWLEDGEMENT

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REFERENCES

1. Laitinen, H. A.; Harris, W. E.; *Chemical Analysis*, Mac Graw-Hill, N.Y., pp 426-462 (1975).

2. Ito, Y.; Weinstein, M.; Aoki, I.; Harada, R.; Kimura, E.; and Nunogaki, K.; *Nature*, **212**, 985 (1966).

3. Mandava, N. B.; Ito, Y.; Conway, W. D.; *Am. Lab.*, **14-10**, 62, (1982).

4. Mandava, N. B.; Ito, Y.; Conway, W. D.; *Am. Lab.*, **14-11**, 48 (1982).

5. Special Issue of J. Liq. Chromatogr., Vol. 7, Number 2 (1984).

6. Special Issue of J. Liq. Chromatogr., Vol. 8, Number 12 (1985).

7. Mandava, N. B., *J. Liq. Chromatogr.*, **8**, 2127 (1985).

8. Ito, Y.; Bowman, R. L., *J. Chromatogr. Sci.*, **8**, 315 (1970).

9. Tanimura, T.; Pisano, J. J.; Ito, Y.; Bowman, R. L., *Science*, **169**, 54 (1970).

10. Centrifugal Partition Chromatograph, Technical Information, Sanki Lab., Model LLN, cartridge 250 W.

11. Murayama, W.; Kobayashi, T., Kosuge, Y.; Yano, H.; Nunogaki, K. Y., *J. Chromatogr.*, **239**, 643 (1982).

12. Berthod, A.; Duncan, J.D.; Armstrong, D. W., *J. Liq. Chromatogr.*, **11** in press, (1988).

13. Berthod, A.; Armstrong, D. W., *J. Liq. Chromatogr.*, **11** following paper in this issue (1988).