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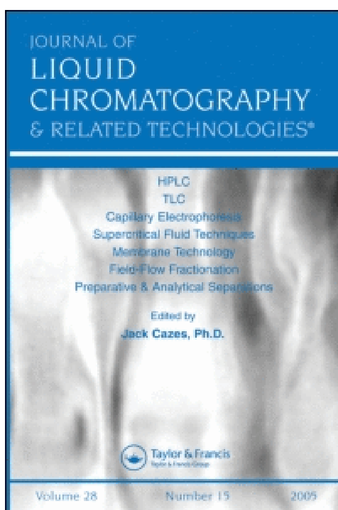

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Centrifugal Partition Chromatography. IV. Preparative Sample Purification and Partition Coefficient Determination

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CENTRIFUGAL PARTITION CHROMATOGRAPHY. IV. PREPARATIVE SAMPLE PURIFICATION AND PARTITION COEFFICIENT DETERMINATION

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ABSTRACT

Centrifugal Partition Chromatography (CPC) uses a liquid as a stationary phase. This liquid is held in channels by a centrifugal field. The ability of CPC to purify a sample was tested using a mixture of 2,2 p-chlorophenyl-1,1,1-trichlorethane (DDT) and the corresponding dichlorethane (DDD). It is shown that, in one run, up to 675 mg of DDT can be purified at 100%, with a recovery ratio of 100% (baseline restoration between peaks) using the liquid system methanol (mobile phase)-hexane (stationary phase). The factors limiting the mass load are (i) the selectivity or partition coefficient difference between the solutes, (ii) the efficiency and (iii) the solute solubility in either of the phases. The mass load must be measured first by increasing the sample concentration of the injected solution, then by increasing the injection volume up to the point where peak overlap occurs. It is theoretically demonstrated that CPC can be used to determine partition coefficients over a five order of magnitude range, from 0.003 to 340, by adjusting the stationary phase volume.

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INTRODUCTION

In the first paper of this series, we presented the general features of centrifugal liquid chromatography (CPC). This technique is closely related to other countercurrent chromatography techniques (CCC). CPC does not employ a solid support matrix, but it uses two immiscible liquids, one as the stationary phase, the other as the mobile phase. The general features of CPC are mainly those of CCC (2-5) As there is no solid stationary phase, all complications due to silica are avoided: there is no excessive adsorption and/or denaturation of samples, no contamination, no pH limitation, no peak tailing due to solute interaction with residual silanols.

Most of CCC apparatuses use a centrifugal field to maintain the liquid stationary phase while the mobile phase is pumped through. The Ito coil-planet centrifuge, the helix CCC, and the angle rotor CCC (2) use a centrifugal field. The droplet CCC does not use such a field. It is based on the passage of small droplets of mobile phase through a series of vertical tubes that hold the immiscible stationary phase (2). The locular CCC device contains small cells in rotation, it corresponds to a droplet CCC system with a centrifugal field that holds the liquid stationary phase (6). As shown in our previous article (1), CPC uses also a centrifugal field to hold the liquid stationary phase while the mobile phase is pumped through in the form of droplets. The aim of this article is to present an evaluation of the capabilities of CPC to purify samples and to determine partition coefficients. The different modes of operation, namely the ascending mode, the descending mode and the recycle mode will be described.

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EXPERIMENTAL SECTION

1-Apparatus

The experiments were performed using a Sanki centrifugal partition chromatograph, 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. Each cartridge, model 450 W, contains 400 channels of 50 uL as described in a previous paper (1). A LC pump (Shimadzu, model LC-6A) and a UV-vis detector (Shimadzu, model SPD-6A) with a preparative cell were connected to the CPC system to circulate the liquids and to detect the solutes, respectively.

2-Chemicals

Two very different liquid systems were used. The first system was methanol-hexane. Those two liquids have low density (0.791 and 0.661 g/cm^3 at 20°C, respectively) and low viscosity (0.54 and 0.29 cP at 25°C, respectively) and have a mutual solubility so high that it was necessary to add 1% v/v of water in methanol to decrease the hexane solubility and 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°C) and a relatively low density (0.845 g/cm^3 at 25°C). The first system was chosen for its convenience to handle: the phase saturation process is fast and the two phases have a relatively similar polarity thereby producing low values for the partition coefficients. The second system was chosen because it is the reference system for the study of the "hydrophobic bonding" of active drugs in biochemistry and the pharmaceutical chemistry. The physico-chemical properties of those systems were discussed previously (7).

The solutes 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°C, the rotor of the CPC apparatus being enclosed in a constant temperature box.

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PREPARATIVE PURIFICATION OF A SAMPLE

Purification of a compound by use of preparative liquid chromatography (PLC) is very often performed when a high purity is required (8). When such a purification is used, the highest possible mass of compound with the highest degree of purity must be obtained in one run.

In classical PLC, the stationary phase is most often bare silica, and the mobile phase is an apolar liquid (normal PLC). It is possible to obtain bonded silica to perform PLC with polar mobile phase (reverse phase PLC). However, the cost of such columns remains very high. In both cases, normal or reverse PLC, the stationary mass overload is the factor limiting the amount of sample loaded in one run.

Mass or volume overload induce a dramatic decrease of the efficiency, resulting in peak overlaps which decrease the amount and/or the purity of recovered compounds. Cretier and Rocca derived a theoretical model able to predict the maximum sample load in a given preparative column packed with silica based stationary phase (9-11). The injected sample size can be raised in two ways: (i) increasing the sample concentration with a constant injection volume (mass load conditions) or (ii) increasing the injection volume of a sample at constant concentration (volume load conditions). Both methods are utilized in the following study.

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1-Mass Load

Table I presents the results obtained by loading in the CPC apparatus increasing amounts of three solutes: 1-naphthol, DDD and DDT. A constant volume (2 ml) of sample dissolved in the mobile phase (methanol) was injected using a 3 mL loop and injecting for 40 s since the flow rate was 3 mL/min.

It can be seen that the efficiency remains remarkably constant when high concentrations of solutes were injected. As shown by Figure 1, there is no tailing on any peak, that means there is no mass overload. This absence of mass overload is due to the liquid nature of the stationary phase. In classical PLC, the stationary phase volume that is effectively used for solute partition or adsorption, corresponds to a small percentage of the whole stationary phase volume. This small usable volume is rapidly saturated as the solute concentration in the mobile phase increases (9-11). Then, to purify mg amounts of sample, huge preparative columns are necessary. In CPC, unlike any solid stationary phase, the whole liquid stationary phase is available for solute exchange. Mass overload will occur only when the liquid stationary phase is saturated by the solute.

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The concentration of the injected solution, corresponding to Figure 1, was 10 g/L, 12.5 g/L and 25 g/l of 1-naphthol, DDD and DDT, respectively. It was not possible to dissolve 0.5 g of DDT in 10 mL of methanol (water 1% v/v and hexane saturated) to prepare a 50 g/L DDT solution. Since the solubility of DDT in methanol (the mobile phase) is lower than the solubility in hexane (the stationary phase), in the studied case, the factor limiting the mass load is only the DDT solubility in the mobile phase. Figure 1 show the purification of 50 mg of DDT, with a 100% recovery and purity degree, in one run.

2-Volume Load

Once the solute saturation of the mobile phase is reached, it is still possible to increase the sample size by increasing the injection volume. The chromatogram in Figure 2 was obtained after injecting the same mass of each solute as in Figure 1. But the sample concentration was five times lower (2 g/L, 2.5 g/L and 5 g/l for 1-naphthol, DDD and DDT, respectively) and the

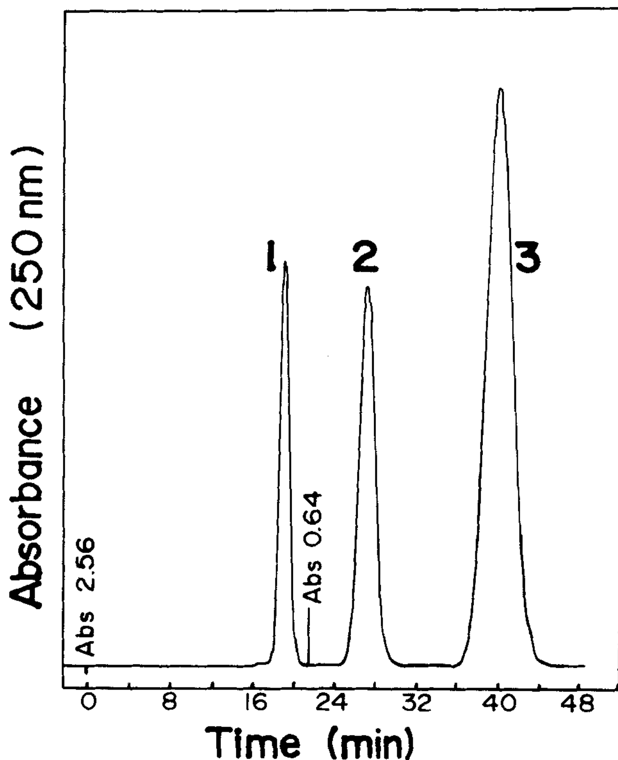

Figure 1: Centrifugal chromatogram obtained with a 2 mL injection volume. Peak 1: 1-naphthol (20 mg); Peak 2: DDD (25 mg); Peak 3: DDT (50 mg). Absorbance: 2.56, wavelength: 250 nm; spin rate: 1300 rpm; flow rate: 3 mL/min; see Table I for all other experimental conditions.

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injection volume and time were five times higher (10 mL and 3 min 20 s) than the corresponding values for Figure 1.

Both Table I and Figure 2 show that the increase of injection volume produced a decrease in separation efficiency. For Figure 2, the peak widths at the peak base (4 σ) are 14.4 mL, 15.6 mL and 18.7 mL for 1-naphthol, DDD and DDT, respectively. The corresponding values in Figure 1 are 5.4

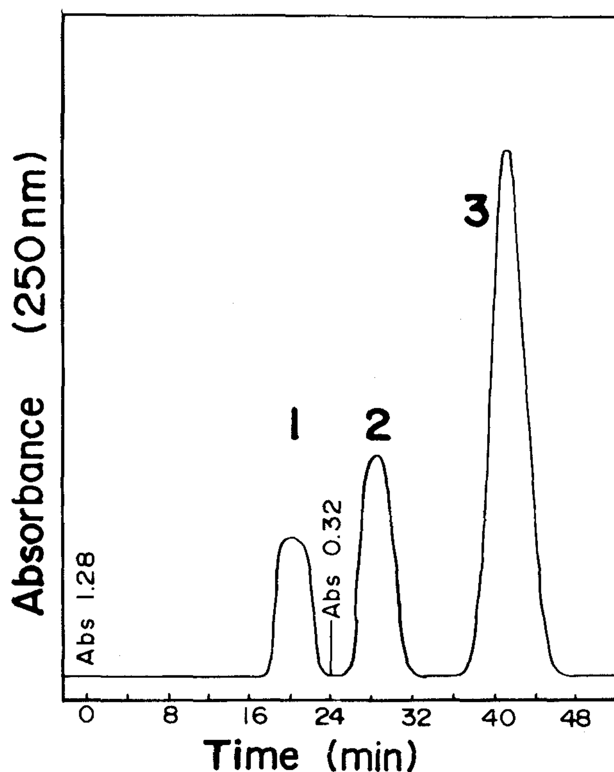

Figure 2: Centrifugal chomatogram obtained with a 10 mL injection volume. Experimental conditions of Figure 1 and Table I, but absorbance: 1.28.

mL, 8.9 mL and 15 mL. As in traditional liquid chromatography, the injection volume produces a band broadening particularly important for the *less retained solutes*. However, the purity and recovery ratio of each compound are still 100% since there is baseline restoration between each peak.

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TABLE I - Efficiency Evolution with Sample Load

Compound	Injected mass mg	v_r mL	N, plates	channels per plate
	0.08	57.6	1400	1.7
	0.4	57.6	1600	1.5
1-naphthol	2.0	57.6	1700	1.4
P = 0.17	20.0	57.6	1950	1.2
	20.0*	61.0	130	18.5
	0.2	81.5	1300	1.8
	1.0	82.5	1300	1.8
DDD	5.0	81.0	1200	2.0
P = 0.47	25.0	81.5	1300	1.8
	25.0*	86.5	320	7.5
	0.4	120	1050	2.3
	2.0	120	1050	2.3
DDT	10.0	120	1000	2.4
P = 0.94	50.0	120	1000	2.4
	50.0*	125	650	3.7

* 10 mL injection, see text and Figure 2.

Flow rate 3 mL/min., 20°C, 1300 rpm, descending mode, pressure 37 kg/cm^2, six cartridges, 2400 channels, mobile phase: methanol (water 1% v/v), v_o = 43.7 mL, stationary phase: hexane, v_s = 81.2 mL.

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Given the symmetry of the peaks and the absence of mass overload, it is possible to estimate roughly the maximum injection volume, V_{max}, that does not produce any peak overlap, using:

$$v_{max} = v_{r2} - v_{r1} - [(W_2 + W_1) / 2] \quad [1]$$

in which the subscripts 1 and 2 correspond to the two compounds being separated, v_r is the retention volume and W is the peak width at base. In the conditions of our experiments, if 1-naphthol must be separated from DDD with a concentration ratio DDD/1-naphthol of 1.25, the maximum injection volume given by eq. 1 is 17 mL. If DDD must be separated from DDT, with a concentration ratio DDT/DDD of two, the maximum injection volume is 27 mL. Then, the maximum amount of DDT that it is possible to purify, with 100% recovery and purity, in one run is 675 mg (27 mL x 25 mg/mL). If the degree of purity required and/or the recovery ratio are less than 100%, i.e. a certain peak overlap is allowed, depending on the criterion, more than 1500 mg of DDT can be purified in one run with the DDT/DDD ratio of two (10). If DDD is one impurity of DDT (i.e. 2% or less), the DDD peak width at base decreases, eq. 1 shows that the maximum injection volume increases, allowing one to purify a larger amount of DDT in one run.

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3- Recycling Mode

As in PLC, it is possible to recycle the mobile phase with the CPC apparatus. This feature can be used to save expensive solvents or to improve a partial separation. Figure 3 show successive chromatograms produced by recycling. Table II lists the chromatographic parameters corresponding to Figure 3.

After the second recycle pass, fluorene, anthracene and DDT can be recovered with a purity higher than 99% (Figure 3b). Table II shows that the efficiency increases linearly with the number of passes. The number of channels required to obtain one theoretical plate, which is the CPC parameter equivalent to the HETP in classical LC (1), is constant. That means that the extra-rotor band broadening due to the connecting tubing and detector is minimal.

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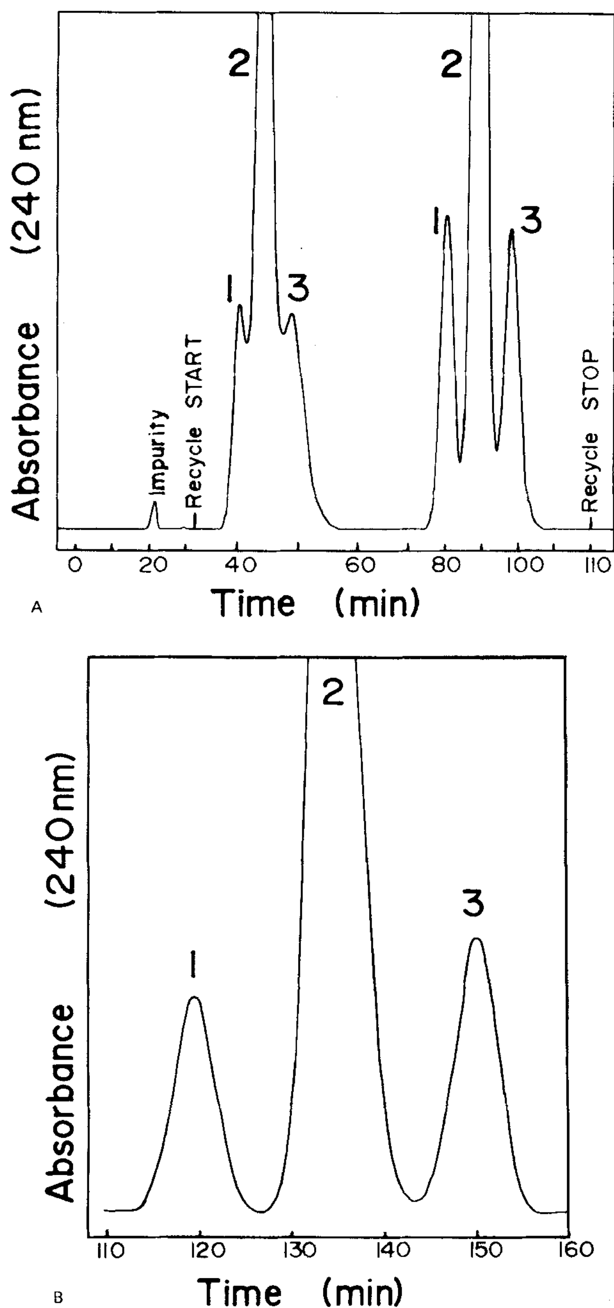

Figure 3: The recycle process. Peak 1: Fluorene (0.72 mg); Peak 2: Anthracene (0.19 mg); Peak 3: DDT (0.60 mg). Absorbance: 0.16, wavelength: 240 nm. **A:** First pass and first recycle. **B:** Second recycle. Experimental conditions in Table II.

TABLE II - Evolution of a Separation While Recycling

Recycle #		Fluorene			Anthracene			DDT	
		v_r mL	N plts **chnl/plt**	R	v_r mL	N plts **chnl/plt**	R	v_r mL	N plts **chnl/plt**
0	a	120	1100 **2.2**	-	133	1200 **2.0**	-	147	1080 **2.2**
0	b	120	-	~0.5	134	-	~0.5	148	-
1	b	240	2000 **2.4**	1.0	267	2000 **2.4**	1.0	294	2400 **2.0**
2	b	360	3000 **2.4**	1.5	405	3040 **2.4**	1.3	452	3900 **1.8**

a) values obtained each solute being injected separately,
b) values corresponding to Figure 3.

v_r = retention volume, N=plate number and **channels per plate** number, R=resolution factor,

Mobile phase: methanol (water 1.1% v/v), v_0=60mL; Stationary phase: hexane, v_s=65mL; 6 cartridges; 2400 channels; 20°C; descending mode; 1300 rpm; 3 mL/min.

The hexane-methanol partition coefficients were 0.92; 1.13; 1.34 for fluorene, anthracene and DDT, respectively.

The recycling process can be used to improve the recovery ratio, however, the best way to enhance a compound purification is to use the versatility of CPC. This technique allows one to modulate not only the mobile phase strength, like in PLC, but also the stationary phase polarity. The partition coefficients of the compounds are likely to evolve in a different way, producing an tunable separation.

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PARTITION COEFFICIENT DETERMINATION

1-Theoretical Considerations

As presented in a previous paper (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.

From Equation 2, one can derive:

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

If the stationary phase volume is known, the measurement of the retention volume of a solute allows one to determine its partition coefficient between the stationary and the mobile phase used.

The stationary phase volume can be determined accurately using the retention volume, v_r', of a "tracer" solute, i.e. either a solute whose partition coefficient is known, then:

$$v_s = [v_r' - v_{t)} / (K - 1)] \qquad [4]$$

or an unretained solute, then:

$$v_r' = v_o \qquad \text{and} \qquad v_s = v_t - v_r' \qquad [5]$$

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2- Modes of operation

Two different modes can be used with the CPC apparatus: the ascending and the descending mode. In the ascending mode, the stationary phase is the lower and denser liquid and the mobile phase, the lighter liquid, is flowed through the stationary liquid from the bottom to the top of the apparatus. The droplets of mobile phase move through the stationary phase against the centrifugal field. In the descending mode, everything is reversed.

The partition coefficient, K, of eq. 2, measures the affinity of the solute for the stationary phase. When the mode is switched from ascending to descending, the partition coefficient, K', obtained in the descending mode, corresponds to 1/K, K being the partition coefficient obtained in the ascending mode.

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3- Partition coefficient range

To determine the actual partition coefficient range, the experimental conditions must be taken in account. The retention volumes can be determined with an experimental error margin of 1% with a minimum error volume of 0.5 mL. Differentiating eq. 3, one obtains:

$$dK / dv_r = 1 / v_s \qquad [6]$$

The relative error is expressed by:

$$dK / K = dv_r / (v_r - v_t + v_s) \qquad [7]$$

Using finite differences (Δ) instead of differentials (d) we get:

$$\Delta K / K = \Delta v_r / (v_r - v_o) \qquad [8]$$

Equation 8 shows that the relative error on K increases dramatically as the solute retention decreases. For example, with 50 mL and 50.6 mL as the dead volume, v_o and the retention volume, v_r of a compound, respectively; the partition coefficient, calculated using eq. 3, is 0.008 with 125 mL and 75 mL as the total volume, v_t, and the stationary phase volume, v_s, respectively. Taking in account the experimental error margin of 0.5 mL on the retention volume, the partition coefficient is somewhere between 0.0013 and 0.0143. As indicated by eq. 8, that corresponds to a relative error as high as 80%. If the partition coefficient K' (= 1/K) is wanted, the measured value is 125 (1/0.008) with a relative error of 560 %. The partition coefficient K' is somewhere between 70 and 770. Such a low accuracy is not acceptable.

If a relative error +/-10% is considered as acceptable, eq. 8 indicates that the retention volume must be at least 5 mL higher than the dead volume *whatever the total volume is*. Then, eq. 3 gives the minimum value of K as:

$$K = 5 / v_s \qquad [9]$$

With six cartridges, an actual stationary phase volume can be 75 mL, and the minimum K value is 0.067. With the relative error of 10%, the minimum value is contained between 0.06 and 0.073.

The higher the retention volume, the lower the error (eq. 7). However, the duration of the experiment will be a limiting factor for the high K value determinations. If 12 hours is considered as the maximum reasonable length of time for an experiment, the maximum retention volume is 4300 mL at a flow rate of 6 mL/min. The maximum K value is given by:

$$K = [(4300 - v_t) / v_s] + 1 \qquad [10]$$

With six cartridges, actual values for the total volume and the stationary phase volume are 125 mL and 75 mL, the highest K value is 57.

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Equation 7 and 8, for the minimum and maximum K values, respectively, both use v_S, the stationary phase volume. One of the advantages of the CPC apparatus used in this study was that it used cartridges. From 1 to 12 cartridges could be loaded in the centrifuge, provided that the rotor is balanced using unconnected cartridges. When only one cartridge was used, the total and stationary phase volumes were 20.8 mL and 12.5 mL, respectively. With such conditions, the partition coefficient range was from 0.4 to 340.

Table III gives the ranges of solute partition coefficients between the lighter liquid A (for example, octanol) and the heavier liquid B (water for instance) that can be obtain in different system configurations (i.e., cartridge numbers).

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The overall range is from 0.003 to 340, which is five orders of magnitude. The log K range lies from -2.5 to +2.5. Table III shows that the low values of K will be accurately determined in the ascending mode, with Liquid A as the mobile phase. On the other hand, the high values of K will be determined in the descending mode, Liquid B being the mobile phase.

As demonstrated in a previous work, the stationary phase volume, v_S, has a ruling effect on efficiency and selectivity (12). Reducing the stationary phase volume reduces the retention times (eq. 2), but it reduces the efficiency and selectivity as well. Figure 4 presents the determination of the octanol-water o-chlorophenol partition coefficient. Phenol has been added as a "tracer". The phenol retention volume, 262.5 mL, allows one to calculate the stationary phase volume, v_S = 7.9 mL, with 29 and 41.6 mL as the octanol-water phenol partition coefficient and the total system volume (two cartridges), respectively (eq. 4). The retention volume of o-chlorophenol is 1170 mL (+/- 4 mL). The o-chlorophenol partition coefficient is 143.8 (+/- 0.5), log K = 2.158 (+/- 0.001). The efficiency of the phenol peak is only

TABLE III - Partition Coefficient Range

Number of Cartridges	Number of Channels	v_t mL	v_s mL	DESCENDING K_{min}	DESCENDING K_{max}	ASCENDING K_{min}	ASCENDING K_{max}
1	400	20.8	12.5	0.4	340	0.003	2.5
2	800	41.6	25	0.2	171	0.006	5
4	1600	83.3	50	0.1	85	0.012	10
6	2400	125	75	0.07	57	0.018	15
8	3200	167	100	0.05	42	0.024	20
10	4000	208	125	0.04	34	0.030	25
12	4800	250	150	0.03	28	0.035	30

(mode *)

*In the descending mode, Liquid B (water) is the mobile phase, in the ascending mode, Liquid A (octanol) is the mobile phase.

K_{min} corresponds to a minimum retention volume 5 mL higher than the dead volume (relative error +/-10%, eq. 7).

K_{max} corresponds to a maximum retention volume of 4300 mL (12 hours at 6 mL/min.)

eight plates (100 channels per plate) and the efficiency of the o-chlorophenol peak is seven plates (115 channels per plate) showing the dramatic effect of the low stationary phase volume on both the selectivity and the efficiency (compare with Figure 1). When using this mode, peak overlap is a problem. Therefore, partition coefficient determinations must be done injecting only one compound at a time.

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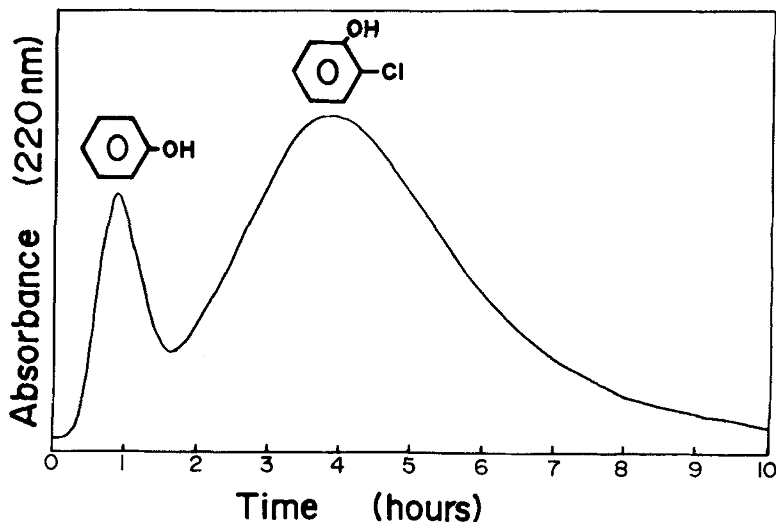

Figure 4: Chromatogram of phenol and o-chlorophenol for partition coefficient determination. Mobile phase: water, Stationary phase: octanol, descending mode, 5 mL/min., two cartridges, 800 channels, 900 rpm, absorbance: 0.08, Wavelength: 220 nm, 25°C.

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The practical feasibility of the theoretical study presented here will be done in the case of the octanol-water partition coefficient in the following paper of this series.

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