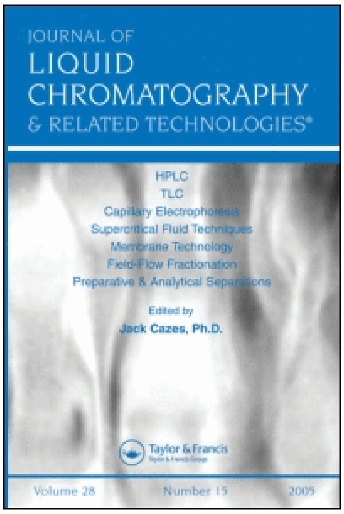

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CENTRIFUGAL PARTITION CHROMATOGRAPHY. V. OCTANOL-WATER PARTITION COEFFICIENTS, DIRECT AND INDIRECT DETERMINATION

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ABSTRACT

Octanol-water partition coefficients (K_{oct}) are one of the accepted physico-chemical parameters for predicting the biological effects of organic chemicals. It is demonstrated that centrifugal partition chromatography (CPC) can be used to determine directly the K_{oct} values from about 0.003 to 300. The mobile phase must be water and the stationary phase must be octanol for accurate determination of the larger K_{oct} values. To reduce retention times and volumes, the stationary phase volume can be decreased. This can be done using an original mode: the "underload" mode. The "underloading" procedure is described. To extend the K_{oct} range which can be determine by CPC, the system hexane (60%) octanol (40%)-water was used. A single linear relationship between log K in this system and log K_{oct} was established allowing to determine the K_{oct} values up to 1000 (log K_{oct} = 3).

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INTRODUCTION

The use of n-octanol water partition coefficients (K_{oct}) has been used in predicting the biological effects of organic chemicals from physical properties through the use of quantitative structure reactivity relationships (QSAR). In water research, the log K_{oct} value has become a critical physical property for predicting toxicity to aquatic organisims, bioconcentration for fish and other organisms in the food chain (1). K_{oct} values are used by the Food and Drug Administration and the Environmental Protection agency as a physical parameter to estimate the tendency of an organic chemical to bioconcentrate in living cells (2).

If the molecular structure of a compound is known, the K_{oct} value can be determined theoretically using the Hansch (3) or the Free-Wilson (4) approaches. Both methods assumes that each substituent of a given molecule plays a constant and additive role on the K_{oct} value, this being true from compound to compound. The Free-Wilson method is oriented toward the biological activity of drugs. Those methods give a good approximation of the K_{oct} values for a given molecule without experimentation. They are extensively used in QSAR studies (5). However, for the same functional groups, the substituent constant approach is not yet completely reliable. Moreover, the structure of the chemical must be known, which precludes these approaches from use in assessing K_{oct} values of uncharacterized mixtures.

The best experimental determination of K_{oct} values is the classical shake-flask method (2). But this method has several limitations: (i) chemical with K_{oct} values higher than 1000 (log $K_{oct} > 3$) cannot be determined with the same precision as chemicals that distribute more evenly between phases, (ii) mixtures of chemicals cannot be used. (iii) For a high K_{oct} value determination, large amounts of pure compound are needed. Different improvements to the shake-flask method were proposed. By using radiolabeled compounds, it is possible to extend the highest obtainable K_{oct} value by the shake-flask method to the 10,000 range ($\log K_{oct} = 4$).

Another alternative is to find extra-thermodynamic relationships between K values in different solvent systems. Relationships like:

$$\log K_2 = a \log K_{oct} + b \qquad [1]$$

can be derived. K_2 is the partition coefficient in the liquid system 2 and K_{oct} is the partition coefficient of the same molecule in the octanol-water system. a and b are constants characterizing the solvent system 2 versus the octanol-water system. A log of solvent regression equations were established by Hansch and coworkers (6). If a is lower than 1 and b is negative, the K_2 value is b orders of magnitude lower than K_{oct} and increases according to:

$$K_2 = K^a_{oct} \times 10^b \qquad [2]$$

Unfortunately the correlation coefficients of such relationships often are poor. To obtain good correlation, proton donor solutes must be separated from proton acceptor solutes, and two relationships, with acceptable correlation coefficients, can be derived from these two class of compounds (6).

The use of liquid chromatography (LC) is another convenient , indirect way to determine the K_{oct} values (2, 7-9). The chromatographic log k' values of compounds can be linearly related to the log K_{oct} values using equations similar to eq. 1. The advantages of LC are relative rapidity, low solute consumption and the ability to determine the k' values of compounds in a mixture. The shortcomings are those stated for the different solvent systems because the LC mobile phase is most often a hydro-organic mixture (acetonitrile or methanol-water) (7-9).

Centrifugal partition chromatography (CPC) can be used to measure partition coefficients (10). Using CPC, Terada et al. (11) obtained acetonitrile-hexane and octanol-hexane-water partition coefficients and related these to octanol-water coefficients. In this study the direct determination of octanol-water partition coefficients for 17 compounds using CPC is reported. The study of the octanol-hexane-water partition coefficient has also been done and compared with previous results (11).

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 (10). 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

Octanol was supplied by Aldrich Chemicals (Milwaukee, WI) and used as received. Hexane was from Fisher. All solutes were analytical grade and were supplied by Dow Chemical (Midland, MI).

3-Procedure

In a recent paper, it was shown that the retention volume, v_r, of a compound is linearly dependent on the partition coefficient, K (12):

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

where v_t and v_s are the total internal volume of the CPC apparatus and the stationary phase volume, respectively. The partition coefficient, K, is defined as the ratio of the concentration of solute in octanol over the solute concentration in water. The partition coefficient determination is calculated using:

$$K = [(v_r - v_t) / v_s] + 1 \qquad [4]$$

Eq. 4 show that the stationary phase volume must be known exactly. v_s is determined using an unretained compound with:

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

or using the retention volume, v_r', of a "tracer" solute i.e.,, a solute whose partition coefficient is known, then:

$$v_s = [(v_r' - v_t) / (K - 1)] \qquad [6]$$

We have shown that the partition coefficient range is bounded by two limits: (i) the lower one, obtained setting the lowest acceptable precision on the K value to 10% ($\Delta \log K = +/- 0.03$):

$$K >= 5 / v_s \qquad [7]$$

(ii) the upper limit, obtained setting the highest experiment duration to 12 hours (10):

$$K < [(4300 - v_t) / v_s] + 1 \qquad [8]$$

As the solutes we wanted to investigate had partition coefficient higher than unity, the stationary phase must be octanol and the mobile phase must be water in order to obtain the lowest possible error (10). The pH value of water was adjusted to 4 with hydrochloric acid for the K_{oct} determination of acidic and neutral compounds (benzoic acids, phenols, benzene derivatives). The pH was adjusted to 9 (ammonium hydroxide) for the K_{oct} determination of pyridine derivatives. All solutes were dissolved in octanol prior to injection.

RESULTS AND DISCUSSION

The determination of low K_{oct} values ($K_{oct} < 5$) is easily accomplished using either octanol or water as the mobile phase. The shake flask method also gives very accurate results with low amounts of compounds in this range. Eq. 8 shows that the highest K value obtainable with CPC (given the imposed limits) can be increased by decreasing the stationary phase volume, v_s. There are two ways to decrease this volume: (i) decreasing the number of cartridges loaded in the rotor (see Ref. 10), (ii) using the "underload" mode described below. If the v_s volume is decreased, the resolution factor R is decreased as well:

$$R = \frac{N^{1/2} (K_2 - K_1)}{4(v_o/v_s) + 2(K_2 + K_1)} \qquad [9]$$

K_1 and K_2 are the K_{oct} values of two different compounds, 1 and 2, respectively, N is the plate number (efficiency) of the CPC apparatus. However, if the only goal is to measure partition coefficient and the solutes are injected one at a time, resolution is not an important factor. All that is needed for an accurate partition coefficient is to accurately measure the difference in v_r vs. v_o.

Using only one cartridge, with only 400 channels, the resolution factor was very low because both the stationary phase volume, v_s, and the plate number, N, were low. Furthermore, the stationary phase "bleeding", described previously (12) seemed to be more of a problem. With two cartridges the bleeding and baseline stability were acceptable.

1-The "Underload" Mode

This mode was designed to associate a high efficiency with a low stationary phase volume, producing an acceptable resolution factor (eq. 9). The term "underload" was chosen because the stationary phase volume loaded in the CPC rotor was much lower than the usual v_s amount obtained using the apparatus as directed by the manufacturer.

The "underloading" procedure is described with octanol, the less dense liquid, as the stationary phase, and water, the denser liquid, as the mobile phase. In this case, the normal mode is the descending mode. The apparatus is filled with the stationary phase: octanol. Then the rotor is started at the working spin rate (900 rpm) and, instead of pushing the mobile phase in the descending mode, water is pushed in the ascending mode i.e., in the opposite way. Technically this is supposed to push the entire stationary phase out of the rotor cartridges, but it does not. Due to the centrifugal field and the crooked shape of the channels, a small amount of octanol remains entrapped in each channel as outlined in Figure 1. A graduated cylinder is placed on the outlet of the detector to measure the octanol volume pushed out by the water. When water comes out of the apparatus, the flow rate is stopped but not the spin rate, the mode value is switched from the ascending to the descending mode, and the flow rate is resumed. The rotor is "underloaded" in octanol.

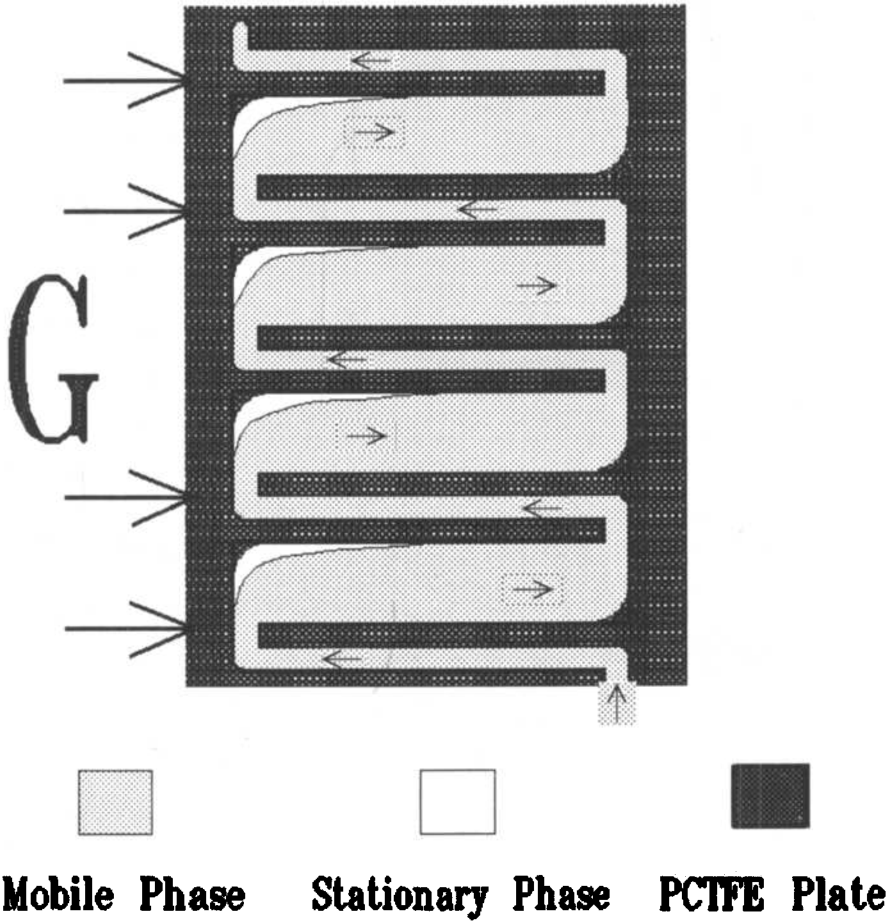

Figure 1: The "underload" mode. Oversimplified view of the possible way octanol is entrapped in the channels. The arrows indicate the flow direction.

Using the procedure, we were able to work with a rotor loaded with only 23 mL of octanol, instead of 75 mL (the actual v_S value obtained under normal conditions) thus reducing the retention volume and time by a factor 3 (~70%). Figure 2 shows the chromatogram obtained when 7 compounds were injected. The resolution of the first two peaks (benzamide and 2-acetoxy benzoic acid) was higher than 1. For peaks 3, 4 and 5 (acetophenone, benzoic acid and o-chlorobenzoic acid, respectively), the resolution was less but the retention volumes (i.e., from the peak maxima) could still be determined with a high precision. The elution of o-chlorophenol and o-chloronitrobenzene gave a broad peak corresponding to the two compounds (Figure 2). However, the resolution was sufficient to obtain the retention volume with an acceptable precision.

The column "bleeding" was an important problem in the underload mode (i.e., the stationary phase volume decreased with time). As described in a previous paper (12), octanol is slowly dissolved by water because the octanol solubility is slightly higher at high pressure (35 kg/cm^2) than at atmospheric pressure. It was shown that v_S decreases linearly versus time. In each injected sample, a stationary phase "tracer" (benzoic acid in the Figure 2 run) was added and the stationary phase volume was corrected according to any detected change. Approximately 1 ml of octanol could be injected into the running apparatus every six hours in order to keep the stationary volume constant. In this way the unit and partition experiments were run continuously for five days without any deterioration in the results. The "column" bleeding can be used to further decrease v_S if very low stationary phase volumes are desired.

2-Direct Determination of K_{oct} Coefficients

Table I lists the partition coefficients obtained for different compounds. Some measures were done with water as stationary phase and

TABLE I - CPC Data for Direct K_{oct} Determination

Compound	Mobile phase[a]	v_o mL	v_s mL	v_r mL	K_{oct}	log K CPC	log K lit.[b]
3-cyanopyridine	W	46.4	78.6	90.8	1.77	0.25	0.36
4-dimethylamino pyridine	O	60	65	96	1.81	0.26	0.39
2-aminopyridine	O	56	69	77.6	3.2	0.50	0.49
hydroquinone	O	50.1	74.9	73.7	3.2	0.50	0.50
benzamide	W	102.3	22.7	202.5	4.4	0.64	0.64
3-hydroxypyridine	O	60.8	64.2	74.4	4.7	0.67	0.48
pyridine	O	58	67	71.2	5.1	0.70	0.65
2-acetylbenzoic acid	W	96.8	28.2	270	6.1	0.79	0.81
	O	46.8	78.2	58.4	6.7	0.83	0.81
o-phthaldiamide	W	100.6	24.4	395	12.1	1.08	-
aspirin	W	99.6	25.4	450	13.8	1.14	1.19
phenol	W	101.6	23.4	780	29.0	1.46	1.47
4-cyanophenol	O	63	62	64.8	35	1.56	1.66
acetophenone	W	102.3	22.5	1005	40	1.60	1.58
benzoic acid	W	99.6	25.4	2040	76	1.88	1.87
2-chlorobenzoic acid	W	102.9	22.1	2250	97	1.99	2.05
2-chlorophenol	W	103.1	21.9	3270	145	2.16	2.15
1-chloro-2-nitro-benzene	W	103.2	21.8	3750	167	2.23	2.24

[a] W = water; O = octanol.

[b] The literature values were mainly from the 5806 K_{oct} values in Ref. 6 and from the Pomona College of Medicinal Chemistry database.

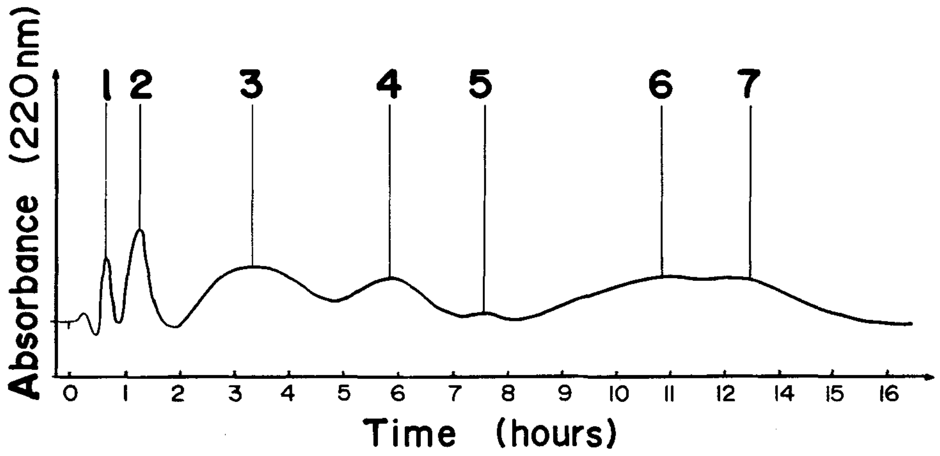

Figure 2: CPC Chromatogram obtained using the "underload" mode. 6 cartridges, v_t = 125 mL, 5 mL/min., stationary phase: octanol, mobile phase: water pH 4, v_s fluctuated from 22.7 mL to 21.7 mL during the 13 hours of the experiment.
1= benzamide; 2= 2-acetoxy benzoic acid; 3= acetophenone; 4= benzoic acid; 5= o-chlorobenzoic acid; 6= o-chlorophenol; 7= o-chloronitrobenzene.

octanol as mobile phase while others were done vice versa. 2-Acetylbenzoic acid had a K_{oct} value of 6.3 that allows a determination by either approach. The retention volume with water as mobile phase was 270 mL (+/- 3 mL, error 1%) that gave 6.1 +/- 0.15 (error 2.5%) for the K_{oct} value. The retention volume with octanol as mobile phase was 58.4 mL (+/- 1 mL, error 2%) that produced 0.148 +/- 0.015 for the water-octanol partition coefficient that is 6.7 +/- 0.9 (error 13%) for the K_{oct} value (Table I). As indicated in a previous paper (10), the K_{oct} determinations are much faster if water is used as stationary phase, but the accuracy becomes very poor for compounds with K_{oct} values greater than 10 or so.

The K_{oct} values, determined directly by CPC, differ from the literature value by about one tenth of a logarithm unit. For ten compounds (over 17), the log K discrepancy is lower than 0.02 logarithm unit. The highest K_{oct} value is 160.7 for o-chloro-nitrobenzene, which corresponded to a retention volume of 3.75 L or a retention time of 12.5 hours at a flow rate of 5 mL/min (Figure 2).

2-Indirect determination of K_{oct} with the hexane-octanol-water system

Terada et al. (11) used CPC to determine octanol-water partition coefficients indirectly (Hansch approach). They made correlations with acetonitrile-hexane and the octanol 20%-hexane 80%-water system. Water was chosen as the stationary phase, which is not the best choice as far as precision is concerned (10).

In order to extend the K_{oct} range which can be determined by CPC, the system of hexane-octanol was also used. The main reason for utilizing an indirect system, specifically the hexane-octanol/water system is that the addition of hexane lowers the K_{oct} to a value more quickly, easily and accurately measured. In this study, the selected composition was hexane 60%-octanol 40% (v/v) as stationary phase and water (buffered at pH 4 or 9) as the mobile phase. Table II shows the experimental data obtained with this liquid system. The log K values were plotted versus the corresponding log K_{oct} obtained from the literature (Figure 3) according to the Hansch method (5,11).

A single linear relationship was obtained regardless of the chemical structure of the investigated compounds:

$$\log K = \underset{(0.230)}{-0.448} + \underset{(0.063)}{0.940} \log K_{oct} \qquad [10]$$

$$n = 21, r = 0.921, s = 352$$

TABLE II-CPC Data for the Hexane-octanol System

Compound	v_o mL	v_s mL	v_r mL	K_{oct}	log K CPC	log K lit.[a]
3-hydroxypyridine	93.4	31.6	107.5	0.44	-0.35	0.46
3-cyanopyridine	94	31	114	0.65	-0.19	0.36
2-acetylbenzoic acid	90	35	151	1.74	0.24	0.81
aspirin	89.7	35.3	232	4.03	0.61	1.19
p-cyanophenol	82.2	42.8	438	8.31	0.92	1.66
nicotine	94	31	375	9.06	0.96	1.17
phenol	85.4	39.6	502	10.5	1.02	1.47
acetophenone	82.8	42.2	1065	23.3	1.37	1.58
o-chlorobenzoic acid	93	32	855	23.8	1.38	2.05
o-bromobenzoic acid	82.2	42.8	1305	28.6	1.46	2.20
benzoic acid	90	35	1125	29.6	1.47	1.87
4-phenylpyridine	120.2	4.8	315	40.6	1.61	2.59
o-chlorophenol	82.8	42.2	2355	53.8	1.73	2.15
2,3-dichloropyridine	94	31	1950	59.9	1.77	2.11
m-methyl benzoic acid	77	48	3300	67.1	1.83	2.37
4(4-nitrobenzyl)pyridine	94	31	4050	128	2.11	2.88
m-chloronitrobenzene	92	33	4425	131	2.12	2.39
p-chlorobenzoic acid	86	39	5325	134	2.13	2.65
2-hydroxy-3,5,6-tri-chloropyridine	120.3	4.7	810	147	2.17	3.21
Diuron	91	34	5700	165	2.21	2.77
2,4 dichlorophenol	83	42	16550	392	2.59	3.06

[a] The literature values were mainly from the 5806 K_{oct} values in Ref. 6 and from the Pomona College of Medicinal Chemistry database.

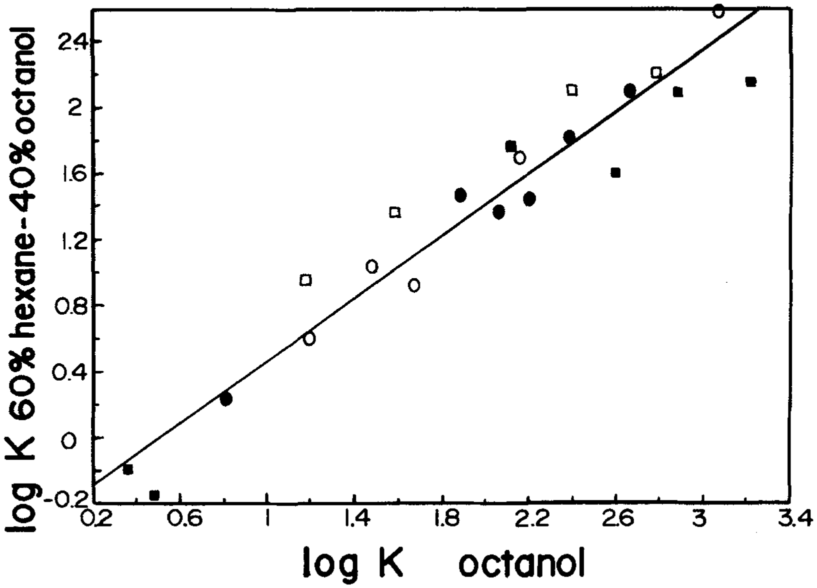

Figure 3: Linear relationship between log K in the octanol (40%) hexane (60% v/v)-water system. The regression line corresponds to eq. 10. ■ : Pyridines; O : Phenols; ● : Benzoic acids; □ : Others.

The values in parentheses under coefficients a and b of eq. 10 are the 95% confidence intervals, n is the number of compounds, r is the correlation coefficient and s is the standard deviation.

The slope of eq. 10 is very close to unity indicating that the solvent environment of the mixture is similar to pure octanol. The intercept (-0.448) shows that the partition coefficients in the hexane-octanol mixture are about 3 times lower than in pure octanol ($10^{-0.448} = 2.8$).

Terada et al. (11) obtained the relation:

$$\log K_{oct} = \underset{(0.642)}{-0.168} + \underset{(0.285)}{1.732} \log K \qquad [11]$$

$n = 14, r = 0.967, s = 0.340$

which can be rearranged as:

$$\log K = 0.097 + 0.577 \log K_{oct} \qquad [12]$$

The slope of eq. 12 is much lower than 1. The solvent environment of the hexane-octanol (80-20% v/v) mixture is closer to pure hexane than to pure octanol. However, the indirectly determined range that they claimed was from 50 to 400,000 (without error analysis). In our case, with the hexane-octanol (60-40% v/v) used as stationary phase, the K_{oct} range indirectly obtained was only from 0.01 to 1150 (Table II). The error on each K value of Table I and II was lower than 10% i.e., the error on each log K value is +/- 0.04 log. unit.

In this study we are certain of the accuracy and precision of the directly determined CPC partition coefficients. It is apparent that most of the deviation between the indirectly determined values and the shake-flask values result from the fact that there is not a perfect correlation (only an approximate one) between the K_{oct} values and the analogous partition coefficients using hexane-octanol/water. This is true of any indirect approach. However, where octanol, octanol-hexane, etc is used as the mobile phase the error is much greater for compounds of moderate to large K-values (> 20 or so). In fact, this error can dwarf that resulting from an imperfect correlation (10).

In conclusion, it must be pointed out that the octanol-water partition coefficient of any compound can be directly determined using CPC with octanol as stationary phase and water as the mobile phase provided that the K_{oct} value is lower than about 300 ($\log K_{oct} < 2.50$). Higher K_{oct} values can be obtained in three ways: (i) by increasing the allowable error and/or experiment time, (ii) by further decreasing stationary phase volume by bleeding and/or redesign of the cartridge channels and (iii) by using the

Hansch approach with solvents different from octanol as stationary phase and water as mobile phase. Those systems lead to relationships that allow one to determine the approximate K_{oct} values over a broader range.

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