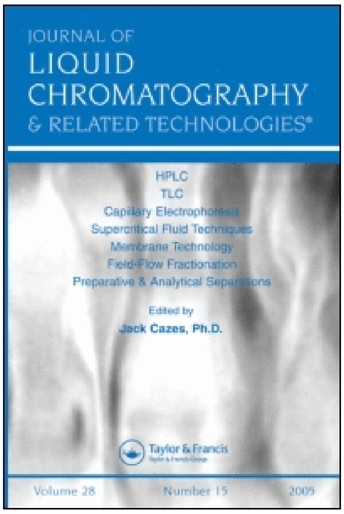

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Berthod, Alain , Menges, Randy A. and Armstrong, Daniel W.(1992) 'Direct Octanol Water Partition Coefficient Determination Using Co-Current Chromatography', Journal of Liquid Chromatography & Related Technologies, 15: 15, 2769 — 2785

To link to this Article: DOI: 10.1080/10826079208016347

URL: http://dx.doi.org/10.1080/10826079208016347

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(15&16), 2769-2785 (1992)

DIRECT OCTANOL WATER PARTITION COEFFICIENT DETERMINATION USING CO-CURRENT CHROMATOGRAPHY

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ABSTRACT

Octanol-water partition coefficients, K_{oct}, can be measured directly using countercurrent chromatography (CCC) with an octanol-water biphasic solvent system. The mobile phase is water saturated with octanol, and the stationary phase is octanol saturated with water. The measurable K_{oct} range was 0.003 to 300. A liquid stationary octanol phase permits the development of a dual-mode elution method using CCC which extends the measurable K_{oct} range to 5,000 (log K_{oct} = 3.7). The co-current CCC method was developed to extend the range to K_{oct} = 20,000 (log K_{oct} = 4.3). In co-current CCC, both the water and the octanol phase move in the same direction at different rates. Using the complete theory that was developed recently (Berthod, A. *Analusis*, 18, 352, **1990**), it was possible to measure with a hydrostatic CCC apparatus loaded with 2 cartridges (800 channels, 49 mL internal volume) the K_{oct} values 10 solutes. The K_{oct} values ranged from 11 (Phthalimide) to 20,000 (Phenanthrene). The continuous detection problem was solved by the post-column addition of 2-propanol as a solubilizing agent with mild heating. As predicted by the theory, the maximum K_{oct} selectivity depends on the octanol to water flow rate ratio. It was found that the efficiency increases with octanol flow rate and, at a given octanol flow rate, the efficiency increases with the solute retention volume (or solute hydrophobicity). The continuous input of octanol at the head of the CCC apparatus produced an autofocusing effect which limits band broadening and peak tailing of the injected band of solute located in the octanol phase.

Octanol water partition coefficient (K_{oct}) is a physico–chemical parameter used to predict the biological effect of organic chemicals. The Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) use K_{oct} values to estimate the tendency of an organic chemical to bioconcentrate into living cells. A new drug cannot be accepted by FDA and EPA without the K_{oct} parameter (1).

There are many non–experimental methods to estimate K_{oct} with statistical or empirical models if the structure of the solute is known. Indirect chromatographic methods, such as HPLC, have been used to estimate K_{oct} by relating it to retention (k′). The accuracy of these methods is dependent on a set of standard solutes, and correlation can be very poor between different classes of compounds. The classical method to measure directly K_{oct} is with a shake flask. In the shake flask method, the concentration of the solute in each phase is then determined by an analytical technique such as HPLC, GC, UV absorbance, etc... The accuracy and precision of this technique decreases substantially for solutes with a log $K_{oct}>2.0$.

Other methods for measuring K_{oct} have been developed including counter current chromatographic techniques which are able to measure directly K_{oct} solely from chromatographic retention data. Counter current chromatography (CCC) is a liquid–liquid chromatographic technique in which both the mobile phase and the stationary phase are liquids. A dedicated instrument keeps one liquid stationary while the other immiscible liquid is pumped through it (2–5). A solute is retained according to the basic CCC retention equation

$$V_R = V_o + K\,V_S = V_T + (K-1)\,V_S \qquad [1]$$

in which V_R is the retention volume, V_T is the internal volume of the apparatus, V_o is the mobile phase volume inside the CCC apparatus and V_S is the stationary phase volume retained inside the apparatus. K is the solute partition coefficient expressed as the ratio of the solute concentration in the liquid stationary phase to the solute concentration in the mobile phase (4, 5). It was shown (6) and proved (7) that CCC was able to give directly the K_{oct} partition coefficients when octanol was used as the stationary phase and water was the mobile phase. The K_{oct} range was 0.003 to 300 or log K_{oct} ranging from −2.5 to 2.5 (6).

It is of great interest to be able to measure higher K_{oct} values. The dual-mode elution was developed to measure K_{oct} values approaching 10,000 (log K_{oct} = 4) (8, 9). Octanol can be used as the mobile phase instead of water. The dual mode elution consists in pushing first water as the mobile phase. A hydrophilic solute with a high K_{oct} value will travel very slowly down the CCC apparatus. After a large amount of water, V_W, has been pumped through the apparatus, the elution mode is reversed. Octanol becomes the mobile phase. An octanol volume, V_{oct}, is pumped in the opposite direction to elute the lipophilic solute out of the apparatus (8, 9). It was demonstrated that the K_{oct} coefficient depends only on the volumes V_W and V_{oct}

$$K_{oct} = V_W / V_{oct} \qquad [2]$$

The drawback of the dual-mode elution method is that it is difficult to switch the role of the two liquid phase keeping the liquid phase ratio inside the apparatus constant. With K_{oct} = 10,000, the water volume, V_W, should be 10^4 times higher than the V_{oct} volume. Pushing water at 6 mL/min for 28 hours will produce a 10 L V_W, the V_{oct} volume is only 1 mL for the K_{oct} = 10,000 solute. Changes in the mL range in the V_S volume due to phase reversal are very likely. The K_{oct} measurement error can be tremendous.

The dual mode elution method is possible because the stationary phase is a liquid. We recently developed the theory of another method also using the fact that the stationary phase is a liquid: the co-current CCC method (10). In this method, both the water and the octanol liquid phases move in the same direction at different flow rates. Octanol becomes an almost stationary phase because the octanol flow rate must be much lower than the aqueous mobile phase flow rate. The theory allowed a thorough study of the selectivity (=peak separation) of the co-current CCC method. It did not give any information on efficiency (=peak sharpness) (10). In this article, the theory is rapidly recalled and the method is tested with a CCC hydrostatic apparatus to study selectivity and efficiency.

THEORY

In co-current CCC, a biphasic eluent moves in a single direction. The eluent is composed of a water mobile phase portion and an octanol

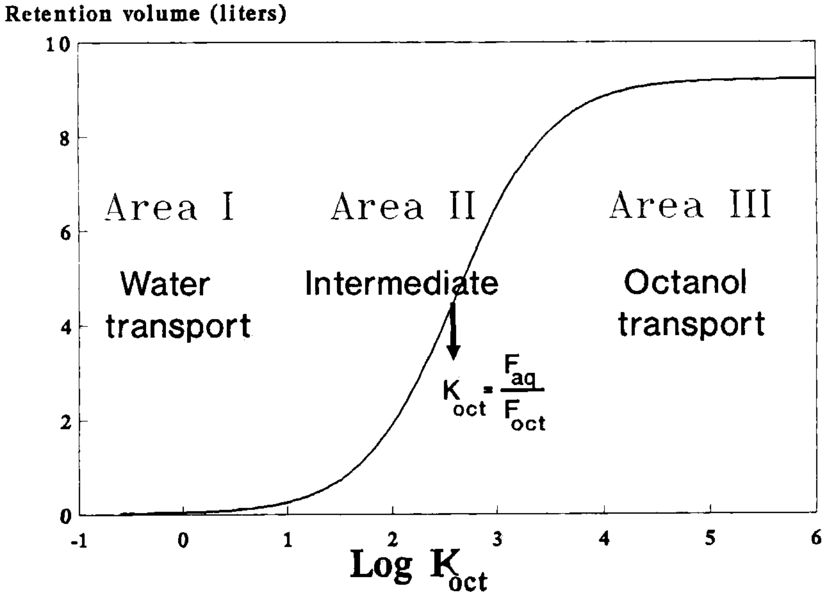

Figure 1: Solute retention volume versus log K_{oct}. The sigmoidal curve shows the three areas (see text). The arrow corresponds to the selectivity maximum, $K_{oct} = 400$, log $K_{oct} = 2.60$. $V_T = 49$ mL, $V_{oct} = 23$ mL, $F_{aq} = 8$ mL/min, $F_{oct} = 0.02$ mL/min.

"stationary" phase portion. The mobile phase velocity is much greater than that of the "stationary" phase. At equilibrium, the ratio of the two portions entering the column is equal to the ratio leaving the column. The aqueous mobile phase flow rate is noted F_{aq}. The "stationary" phase (octanol) flow rate is noted F_{oct}. It was demonstrated that the solute volume retention, V_R, is expressed by (10)

$$V_R = (F_{aq} + F_{oct}) \frac{V_{aq} + K_{oct} V_{oct}}{F_{aq} + K_{oct} F_{oct}} \qquad [3]$$

With $F_{oct} = 0$, the "stationary" phase is static and eq. 3 is equivalent to eq. 1. Figure 1 shows the plot of the retention volume, V_R, versus log K_{oct} for a typical set of flow rate values. A sigmoidal curve is obtained in which three area can be distinguished (10). In Area I, at low K_{oct} values (log $K_{oct} < 1$), V_R increases slowly with K_{oct}. The solute is mainly carried by the aqueous phase. In Area II, at intermediate K_{oct} values depending on the flow rate setting, there is a sharp increase of V_R as log K_{oct} increases. In this area, the solute is carried by both phases. Area III corresponds to lipophilic solutes with high K_{oct} values. They are carried almost exclusively by the octanol phase (10). The retention volume becomes nearly independent of the K_{oct} value (Figure 1). We demonstrated that Area II is centered on the K_{oct} value

$$K_{oct} = F_{aq}/F_{oct} \qquad [4]$$

which corresponds to the maximum selectivity (10).

It was also shown that the retention volume, which is linked to the retention time and analysis duration, could be decreased without any loss of selectivity by reducing the octanol volume, V_{oct}, retained inside the apparatus. Some loss of efficiency is likely (10).

EXPERIMENTAL

Apparatus

The experiments were performed using a centrifugal partition chromatograph (CPC), model CPC-NMF, from Sanki Laboratories Inc., Sharon Hill, Pennsylvania, USA. This apparatus can hold up to 12 cartridges positioned in the rotor of a centrifuge. Each cartridges, model 450 W, contains 400 channels of 50 μL each as described in a previous paper (11). All experiments in this work were done at a rotation speed of 1,000 rpm and with a centrifuge rotor loaded with only two cartridges. This gave an internal CPC volume of 45 mL (V_T = 49 mL adding the 4 mL coil used for detection purposes, see below) and 800 channels. Figure 2 shows a schematic of the co-current CCC set-up. Three LC pumps (Shimadzu, model LC-6A) were used with a controller (Shimadzu, model SCL-6B, not shown in Figure 2). Two pumps were used to push the aqueous mobile phase and the octanol "stationary" phase. The two liquid phases entered through the head (top) of the apparatus (descending mode). The third pump was used for the post-column addition of 2-propanol as described below.

Detection

After column equilibration, two immiscible liquid phases exit the apparatus which renders the effluent turbid, hindering continuous detection. 2-propanol was added post-column as a clarifying agent (Figure 2). A 4 m long teflon tubing coil (volume ca. 4 mL)

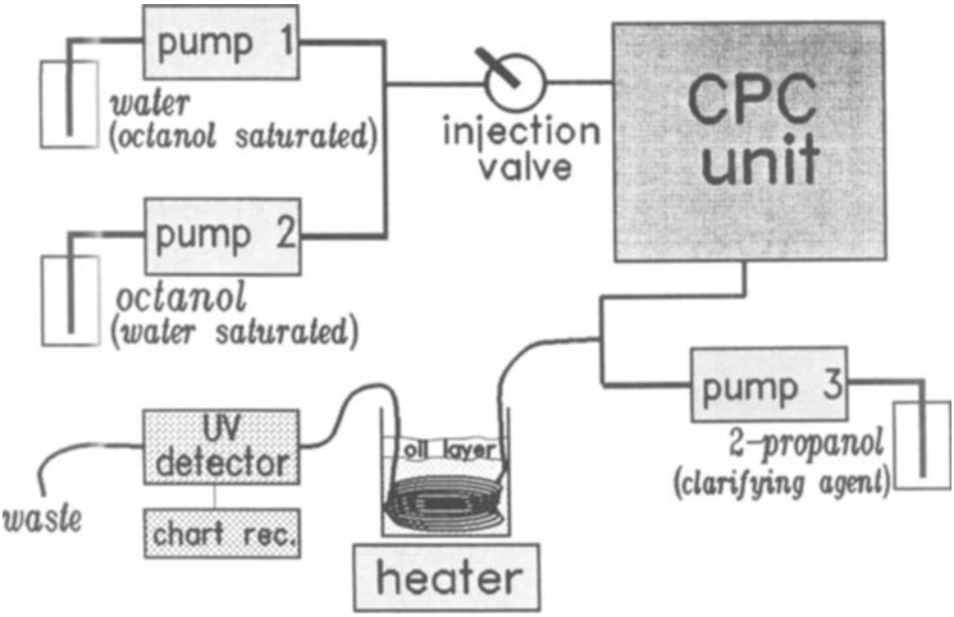

Figure 2: The co-current CCC set-up.

was immersed in a beaker of boiling water to ensure good mixing and to reduce the amount of propanol required to clarify the CCC effluent. An oil layer was added to the water in the beaker to minimize water evaporation (Figure 2). This set-up allowed the CPC apparatus to run with continuous detection for more than 24 hours with a two gallon aqueous phase reservoir. The detector was a Shimadzu SPD-6A UV detector with a preparative 8 μL cell. The detector was set at 210 nm.

Chemicals

Octanol and 2-propanol were purchased from Aldrich Chemicals (Milwaukee, Wisconsin) and used as received. Octanol was always saturated with water and water was saturated with octanol before use. 10 mL of acetic acid were added in each liter of water phase to lower the pH down to ca. 3. The liquid phases were not filtered because octanol is too viscous and because the aqueous phase volumes were too large. The solutes were either donated by Dow Chemical Co. (Midland, Michigan) or purchased from Aldrich.

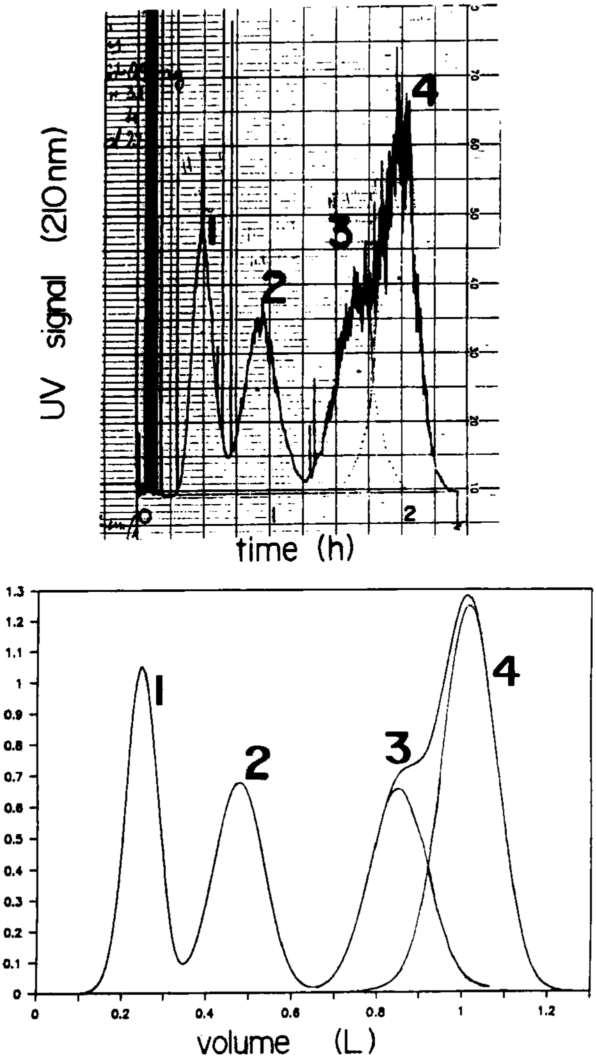

Figure 3: Actual co-current chromatogram (top) and the corresponding smoothed chromatogram (bottom) used for the retention volume measurements. Injected volume: 0.3 mL, **1**-phthalimide (0.9 mg inj.), **2**-p-hydroxybenzoic acid (3.1 mg), **3**-o-chlorophenol (4 mg), **4**-1-naphthol (2.2 mg). F_{aq} = 8 mL/min, F_{oct} = 0.2 mL/min, V_{oct} = 26.3 mL, detection UV, 210 nm, 0.16 a.u.f.s., post-column addition of 2-propanol at 3.1 mL/min.

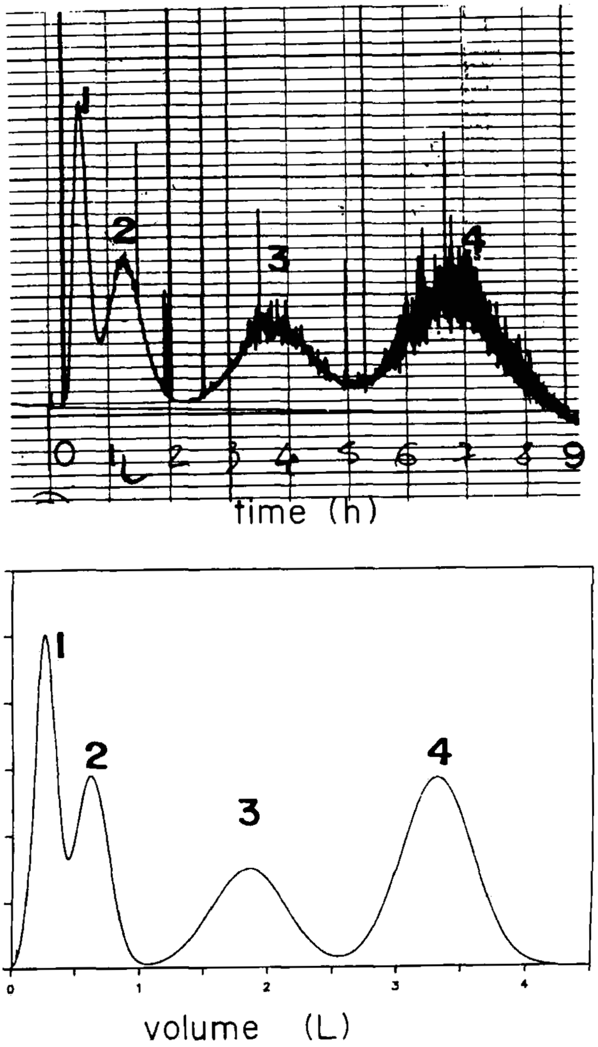

Figure 4: Actual co–current chromatogram (top) and the corresponding smoothed chromatogram (bottom). F_{aq} = 8 mL/min, F_{oct} = 0.05 mL/min, $F_{2\text{-propanol}}$ = 2.4 mL/min, V_{oct} = 23.4 mL, all other experimental conditions were detailed in Figure 2 caption.

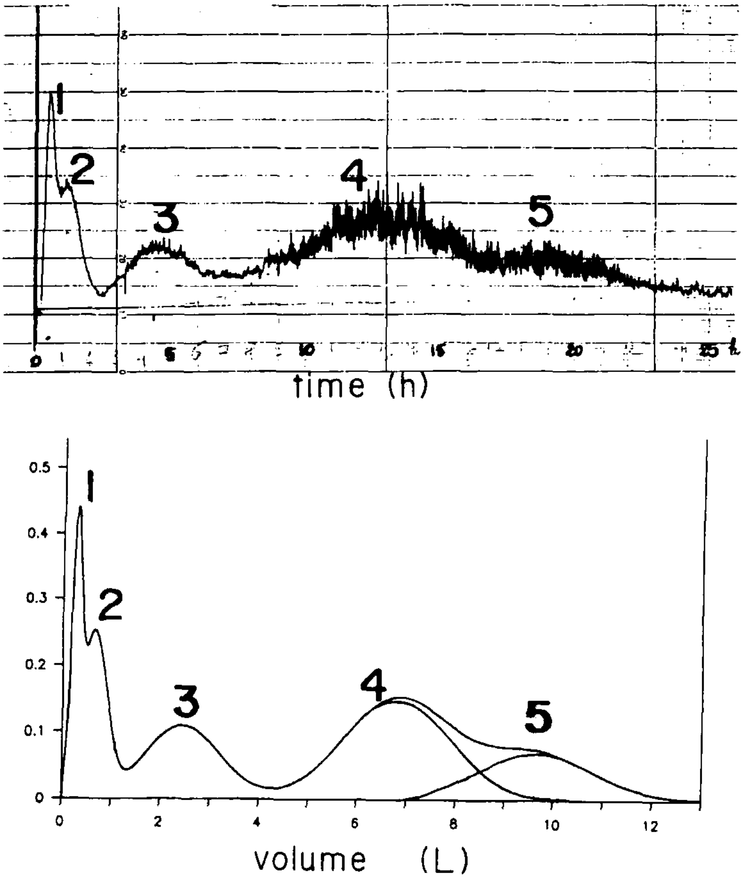

Figure 5: Actual co-current chromatogram (top) and the corresponding smoothed chromatogram (bottom). F_{aq} = 9 mL/min, F_{oct} = 0.02 mL/min, $F_{2\text{-propanol}}$ = 2.7 mL/min, V_{oct} = 22.2 mL, peak 5 is phenanthrene (1 mg injected), all other experimental conditions were detailed in Figure 2 caption.

Table I: K_{oct} and log K_{oct} values obtained for different solutes.

Solute	F_{oct} mL/min	F_{oct} mL/min	V_{oct} mL	V_R mL	t_R h	K_{oct}	log K_{oct} CPC	log K_{oct} litt.
phthalimide	0.02	8	25	287 ±15	0.60	11	1.04 ±0.03	1.15
p-hydroxybenzoic acid	0.02	8	25	653 ±20	1.36	27	1.43 ±0.02	1.58
benzoic acid	0.01	4	29.4	1490 ±30	6.20	57	1.76 ±0.01	1.87
benzene	0.01	4	29.7	2530 ±30	10.51	107	2.03 ±0.006	2.14 ±0.02
o-chlorophenol	0.02	8	25	2720 ±30	5.63	147	2.17 ±0.01	2.17 ±0.02
toluene	0.01	9	20.2	6520 ±50	12.08	500	2.70 ±0.01	2.71 ±0.02
1-naphthol	0.02	8	25	7270 ±50	15.10	1050	3.02 ±0.02	2.98 ±0.04
naphthalene	0.01	9	20.2	15500 ±150	28.63	5100	3.70 ±0.03	3.23 ±0.21
biphenyl*	0.02	8	23.0	8700 ±100	18.10	6700	3.8 ±0.1	3.8 ±0.2
phenanthrene	0.02	9	22.2	9790 ±100	18.08	20,000	4.3 ±0.2	4.4 ±0.3

* experiment done at only 800 rpm rotation speed
V_T = 49 mL, rotation speed = 1000 rpm, 2 cartridges, 800 channels, descending mode, log K_{oct} literature values were obtained from the Pomona College of Medicinal Chemistry log K_{oct} Database.

RESULTS and DISCUSSION

Figures 3-5 show actual co-current chromatograms (top) and the corresponding smoothed digitized chromatograms (bottom). They allowed to obtain the K_{oct} value of five solutes. The K_{oct} values ranged from 11 (phthalimide, first peak) to 20,000 (phenanthrene, last peak of Figure 5). Table I shows the results obtained for these and other compounds.

Retention times and volumes

The retention volume of a solute depends on four parameters: the solute partition coefficient, the aqueous phase flow rate, the octanol phase flow rate and the octanol volume retained inside the apparatus (eq. 3 and Ref. 10). The solute partition coefficient is the parameter we want to measure. The flow rate of each pump can be set at any desired value between 0.01 and 10 mL/min, in 0.01 mL/min increment. The octanol volume retained in the CPC machine is a passive parameter (12). It depends on the centrifuge rotation speed and the flow rates (Table I). It increases with the rotation speed and the octanol flow rate. It decreases when the aqueous flow rate increases. Orthochlorophenol (K_{oct} = 147) was added to all studied solutes or mixtures and used as a tracer to calculate the "stationary" phase volume.

Figures 3–5 and Table I clearly show that retention times and relative octanol and water flow rates are linked as predicted by eq. 3. When different analyses were done at constant F_{aq}/F_{oct} ratio (i.e. same selectivity, eq. 4), the retention volumes obtained for the same compounds were very similar (Table II). Retention times and retention volumes are linked by flow rates. Since retention times decrease when the flow rates increase, the maximum flow rate should be used. However, there are two limitations. First, flow rates are limited by the maximum pressure that the CPC unit can tolerate which is about 60 bars or 850 p.s.i. Second, the selectivity and precision of the K_{oct} determination decreases dramatically as the octanol flow rate increase (10). This point will be further developed thereafter. A high water flow rate has also the advantage to lower the octanol volume, V_{oct}, retained inside the CPC unit. This further reduces the retention volumes and times.

At a constant aqueous flow rate, the retention times and volumes of the high K_{oct} solutes belonging to Area III (Figure 1) are directly proportional to the octanol flow rate as illustrated by Naphthol in Table II. The retention times and volumes of Area I solutes with low K_{oct} values are not modified by octanol flow rate changes. They are carried essentially by water. This is illustrated by Phthalimide in Table II. Does this mean that a high octanol and a high aqueous flow rates will make high K_{oct} measurement fast and easy? Unfortunately not; for hydrophobic solutes the method precision and retention times are linked.

Table II: Effect of flow rates on retention volumes and times and efficiency

	solute	phthalimide $K_{oct}=11\pm1$		p−hydroxybz ac. $K_{oct}=27\pm3$		o−chlorophenol $K_{oct}=147^{c}$		1−naphthol $K_{oct}=1050\pm50$		
F_{aq} mL/min	F_{oct} mL/min	V_R mL	N plates	V_R mL	N plates	V_R mL	N plates	V_R mL	N plates	t_R h
2	0.05	279	25	534	40	981	70	1180	300	9.6
4	0.1	286	30	521	50	928	110	1080	300	4.4
8[a]	0.2	247	40	476	65	850	160	1015	250	2.1
8	0.15	265	40	523	50	1034	70	1305	160	2.7
8	0.1	256	25	552	25	1330	50	1863	110	3.8
8[b]	0.05	257	10	617	20	1850	40	3310	140	6.9
8	0.02	287	7	653	7	2720	15	7270	30	15.1

V_T = 49 mL, 2 cartridges rotating at 1000 rpm (800 channels), descending mode.

a) chromatogram Figure 3

b) chromatogram Figure 4

c) stationary phase volume, V_{oct}, tracer, the value K_{oct} = 147 was used to calculate V_{oct}.

Method precision

The error on the K_{oct} determination depends critically on the aqueous and octanol flow rates. The ratio d(log K_{oct})/dV_R can be used to estimate the method error. We demonstrated that d(log K_{oct})/dV_R was expressed by (10)

$$\frac{d(\log K_{oct})}{dV_R} = \frac{(F_{aq}+K_{oct}F_{oct})^2}{2.3K_{oct}(F_{aq}+F_{oct})(V_{oct}F_{aq}-V_{aq}F_{oct})} \qquad [5]$$

Figure 6 shows the eq. 5 function plotted vs log K_{oct} with F_{aq} = 8 mL/min and six different F_{oct} values. We arbitrarily set the maximum acceptable error on a log K_{oct} determination at ± 0.2 log units. Then, the maximum K_{oct} value which can be measured by the co−current CCC method depends on the error on the retention volume measurement. For example, the phenanthrene retention volume was 9790 mL ± 100 mL. With F_{aq} = 9 mL/min and F_{oct} =

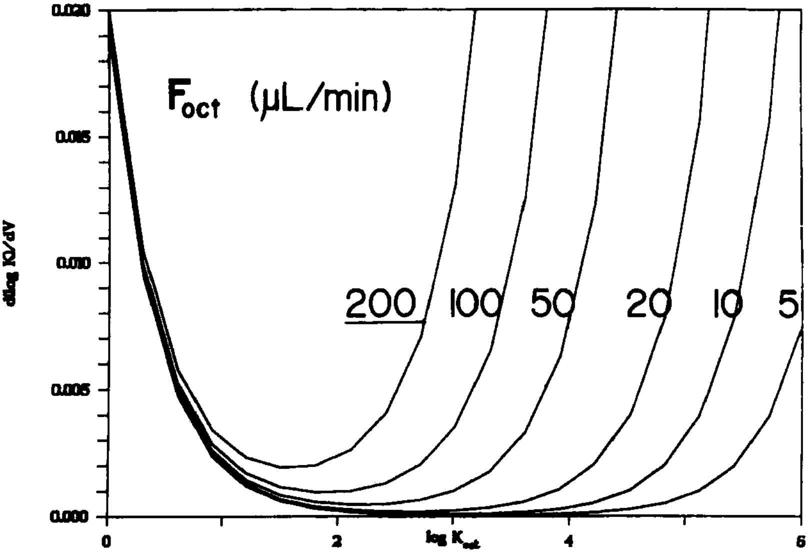

Figure 6: Theoretical curves of the error function plotted versus log K_{oct} for different octanol flow rates. F_{aq} = 8 mL/min. The octanol volume was kept constant: V_{oct} = 23 mL, it was observed experimentally that V_{oct} increased with F_{oct}.

0.02 mL/min, eq. 5 gives d(log K_{oct})/dV_R = 0.0019 log units per mL. The error on the log K_{oct} value of phenanthrene is 100x0.0019 = 0.19 log units (Table I). One should note that the result, 4.3 ± 0.2, for the log K_{oct} phenanthrene value means that the K_{oct} value of phenanthrene is somewhere between 12500 (log K_{oct} = 4.1) and 32,000 (log K_{oct} = 4.5). Figure 6 shows that the error on log K_{oct} is minimum for the solutes that are transported by both liquid phases, i.e. Area II solutes (Figure 1). The error is large for hydrophilic solutes due to their low retention volumes. The error becomes large for hydrophobic solutes depending on the octanol flow rate since the Area III location depends on octanol flow rate (eq. 4). The minimum error area is greatly increased when the octanol flow rate is decreased at constant high aqueous flow rate (Figure 6). Table III lists the maximum K_{oct} value that can be measured with the assumption that the retention volume error is ±1%. With the maximum aqueous flow rate, 9 mL/min, and the minimum octanol

Table III: Maximum measurable K_{oct} values with co–current CCC

F_{oct} μL/min	V_R L	T_R h	K_{oct}	log K_{oct}
200	0.9 ± 0.01	1.9	1,700	3.2
100	1.8 ± 0.02	3.7	3,500	3.5
50	3.6 ± 0.04	7.5	7,000	3.8
20	9 ± 0.1	18.8	18,000	4.25
10	18 ± 0.2	37.5	35,000	4.5
5	36 ± 0.4	75	70,000	4.8
10*	20 ± 0.2	37.5	40,000	4.6

*) F_{aq} = 9 mL/min. Theoretical values obtained with eq. 5 with F_{aq} = 8 mL/min except for the last row, 2 cartridges, V_T = 49 mL, V_{oct} = 23 mL, descending mode, Δlog K_{oct} = ± 0.2.

flow rate, 0.01 mL/min, the maximum measurable K_{oct} value is 40,000, with log K_{oct} = 4.6 ± 0.2 and d(log K_{oct})/dV = 10^{-3} log units per mL (Table III).

Detection

Figures 3–5 show three actual chromatograms (top). In all experiments, we observed a low UV signal noise before injection and at the beginning of a chromatogram. The UV signal noise increased as the chromatogram developed. It was maximum when the last peak eluted and decreased after the last peak (Figure 5, top). This noise is due to the fact that the solutes are not evenly distributed in the mobile phase. As the chromatogram develops, the eluted solutes are more and more hydrophobic. This means that the solutes reside more and more in the octanol phase. The octanol phase comes out of the CPC apparatus in little droplets with some bursts due to pump pulsations or rotor vibrations. The post–column addition of 2–propanol and heat (Figure 2) dissolve the octanol phase and the aqueous phase in one homogeneous phase in which the solute is not evenly distributed. When an octanol burst occurs, there is a local excess of octanol which does not produce noise in the UV detector because octanol does not absorb at 210 nm. When octanol contains an UV absorbing solute, the local excess of octanol is also a local excess of solute which produces a spike in the UV signal.

A HPLC pulse dampener placed before the injection valve did not help to reduce the noise. Such devices need high pressures to be efficient. The CPC working pressures, in the 30 bars or 400 p.s.i. range, were too low. Since the octanol volume needed for an experiment is low (about 30 mL), a syringe pump could be used to push the octanol phase evenly and reduce the UV signal noise. However, there is no way to reduce the vibrations due to the centrifuge rotation. Electronic smoothing by signal processing was the best way we found to measure the retention volumes with the minimum error (Figures 3–5, bottom).

Efficiency

In previous works with octanol and water, we obtained very low efficiency (˜10 plates or less, 80 channels per plate or more) (6, 13, 14). Furthermore, the efficiency decreased when the K_{oct} values (and retention volumes) increased (6, 14). Figure 3–5 and Table II show that the opposite trend was obtained with co-current CCC: the peak sharpness or efficiency increased with retention volume. In the conditions of this work, the highest efficiency that could be obtained theoretically was 800 plates or 1 channel per plate (13). Efficiencies in the 300 plate range (2.7 channel per plate) were obtained for hydrophobic solutes (Table II). The efficiency increased as the octanol flow rate increased. It seems that the continuous introduction of octanol at the system head pushes the hydrophobic solutes, producing some autofocusing of the injected band which reduced band broadening and peak tailing. The observed results is narrower bands or sharper peaks and efficiencies that increase with both the octanol flow rate and the hydrophobicity of the solute (Table II).

A higher efficiency allows higher precision on the peak retention volume measurement. Therefore, it exists an optimum octanol flow rate below which the efficiency decrease offsets the selectivity increase. Given the retention times listed in Table III and the experimental efficiencies obtained (Table II), the optimum octanol flow rate necessary to measure the highest K_{oct} value with the co-current CCC method is 10 μL/min. With 9 mL/min as the aqueous flow rate, the highest measurable K_{oct} value is 40,000 (log K_{oct} = 4.6). When one wants to measure lower K_{oct} values, a higher octanol flow rate can be used. The loss of selectivity will be partially compensated for by the higher efficiency, producing a lower error on the retention volume. The K_{oct} value will be obtained with an acceptable precision and much more rapidly (Table III). In cases of an unknown K_{oct} value, a rapid screening can be done at both high aqueous phase flow rate (8–9 mL/min) and high octanol flow rate (0.2 mL/min). Then, the octanol flow rate can be adjusted to make the K_{oct} measurement with the minimum error.

To conclude this work, we point out that the maximum K_{oct} value that was obtained by co–current CCC is higher than any other CCC method used for such determination. This method is relatively easy to use. The "stationary" octanol phase volume retained in the CPC apparatus is more stable than with the other methods because there is a constant input of octanol. The octanol volume changes due to dissolution as noted in the direct method (6) or to phase reversal in the dual mode method (8, 9) do not exist with the co–current CCC. This method was described for octanol–water partition coefficient determination, but it could be used with other liquid systems in place of phase reversal or gradient elution.

ACKNOWLEDGEMENT

A. B. thanks the Centre National de la Recherche Scientifique (UA 435) for the support of this work. D. W. A. thanks the National Institute of General Medical Science (BMT 2R01 GM36292).

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