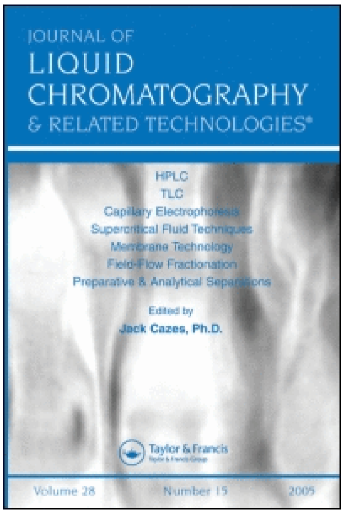

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EXTENDED OCTANOL-WATER PARTITION COEFFICIENT DETERMINATION BY DUAL-MODE CENTRIFUGAL PARTITION CHROMATOGRAPHY

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ABSTRACT

The range of determination of octanol-water partition coefficients by CPC has been extended by the dual-mode approach. In this mode, the analyte was pumped in the descending mode with water as the mobile phase for a predetermined time. The mode was then switched to ascending with octanol as the mobile phase. Lipophilic materials only moved partially through the system in the descending mode. They rapidly eluted in the ascending mode with good signal-to-noise. The partition coefficient was shown to equal the volume of water pumped in the descending mode divided by the retention volume in the octanol phase ascending. A systematic positive error resulted when the experimentally selected descending volume was too small.

INTRODUCTION

Centrifugal partition chromatography (CPC) has been previously shown to give a direct determination of octanol-water partition coefficients (K_{ow}) (1,2). This procedure involved using water-saturated octanol as the stationary phase and octanol saturated water as the mobile phase. The centrifugal field held the octanol stationary phase in 2400 50-μL stages through which the aqueous mobile phase flowed. From the retention volume of the unknown compound, the stationary phase volume and the mobile phase volume, the K_{ow} was calculated. The determination of the K_{ow} was independent of the structure of the unknown because the retention volume was shown to be fully explained by theory. The procedure had an upper limit of 300 in the K_{ow}. This was due to lipophilic compounds typically having a low solubility in the aqueous phase and the broadness of late eluting peaks limiting the detectability. A solution to this problem is to apply a common operation used in CPC called "dual-mode" CPC (3-6). After a predetermined time of pumping in the descending mode, the mode is switched to ascending and the compounds of interest elute in the octanol. The advantage of dual-mode CPC is that the analyte only has to move a small distance through the CPC for a partition coefficient to be determined. Therefore, the determination range of partition coefficients can be extended. In addition, since the analyte remains largely in a small volume of the octanol phase, it is easily detectable.

THEORY

In previous work (1), the determination of K_{ow} was shown to be fully explained by the basic chromatographic theory relating the retention volume (V_r) to K_{ow}, the aqueous mobile phase volume (V_w) and the octanol stationary phase volume (V_o):

Equation 1: $$V_r=K_{ow}*V_o+V_w$$

Therefore, the fractional distance (X) which an analyte migrates through the CPC at a volume of water pumped in the descending mode (V_d) is:

Equation 2: $$X=V_d/(K_{ow}*V_o+V_w)$$

When the mode is switched from descending to ascending, the analyte moves in the reverse direction and from equations 1 and 2 its retention volume in the ascending mode solvent, octanol, (V_{ra}) is:

Equation 3: $V_{ra}=X*V_w/K_{ow}+X*V_o$

The K_{ow} can thus be deduced from equations 2 and 3 to be independent of every parameter except the V_d and the V_{ra}:

Equation 4: $K_{ow}=V_d/V_{ra}$

This derivation assumes that V_o and V_w remain constant. Thus, the flow rate of the octanol in the ascending mode must not exceed that flow rate which forces the displacement of water as the stationary phase from the system; it must be below the flooding flow rate.

EXPERIMENTAL

Apparatus and Method

The system was setup as shown in Figure 1. Typically, a 5-μL aliquot of 1-50% analyte in octanol was injected from the Hitachi model 655A-40 autosampler into the octanol saturated water stream flowing at 4.89 mL/min from the Sanki model LBP-V pump. The flow passed through the 8-port mode valve (Valco Instruments) and into the CPC in the descending mode at 600 rpm. The aqueous phase was recycled. After a predetermined descending flow volume, the mode valve was switched via the data acquisition/control system (Perkin Elmer/Nelson Analytical ACCESS-CHROM) to the ascending mode. In this mode, water saturated octanol was pumped at 0.317 mL/min from a Waters M-45 pump up through the CPC and out through the Linear Instruments model 204 detector with the semi-prep flowcell. The octanol was not recycled. The V_d was measured from the time of

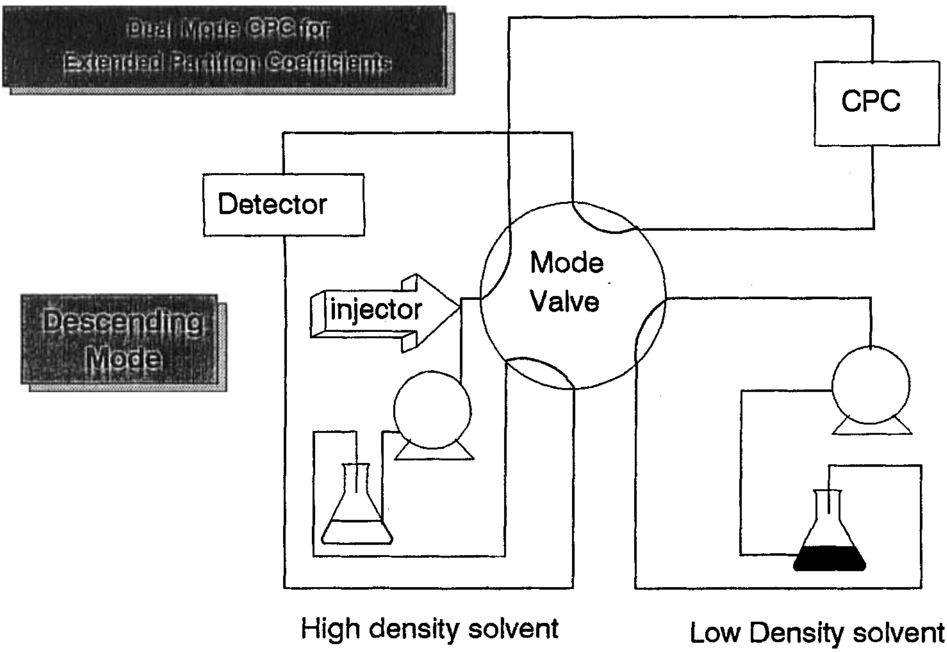

Figure 1. Apparatus for Automated Dual Mode CPC.

sample injection to the time of mode switching. The V_{ra} was measured from the abrupt change in apparent absorbance when the octanol phase entered the flowcell to the manually determined peak maximum of the replotted detector output on a graphics display monitor. Figure 2 is a typical replotted output from the detector.

Test Compounds

The quality of the comparison between literature values of K_{ow} and those determined by this methodology ultimately depend on the variation in the literature values. The results of an OECD Laboratory Intercomparison Testing of the shake-flask method for

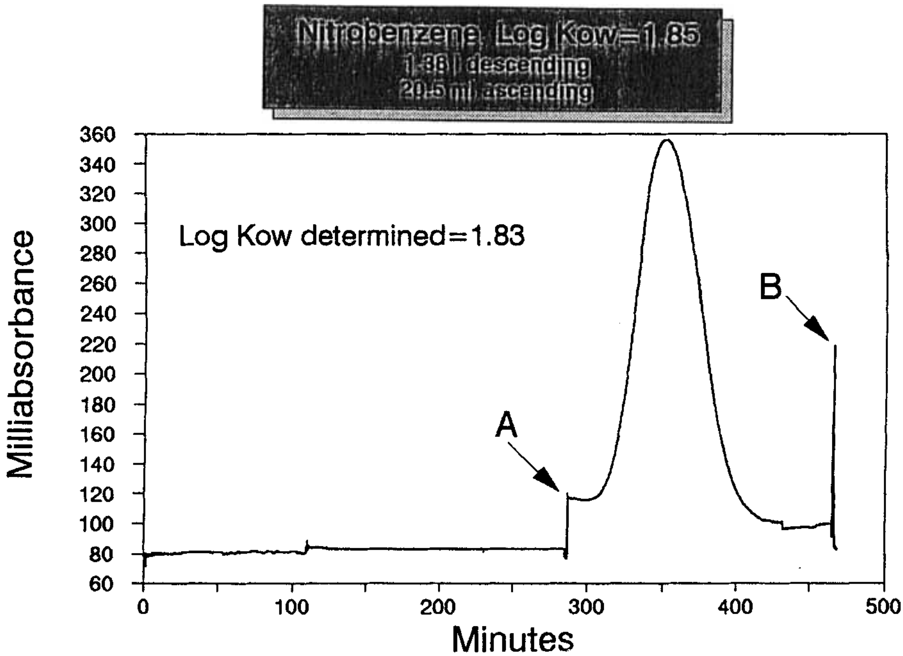

Figure 2. Typical Detector Output Versus Time. "A" marks the switch from descending mode to ascending mode. "B" marks the switch from ascending mode to descending mode.

determining K_{ow} gave an average range of reported log K_{ow}'s for 6 compounds spanning K_{ow}'s of -1.2 to 5.6 of 1.0 (7). Error was not coorelated with the Log K_{ow}. Although this one study cannot be used to assign an error to literature partition coefficients in general, it highlights the potential for error in reference compound Log K_{ow}'s. Table 1 contains the compounds used to test the dual-mode CPC methodology. Included in the table are the number of corraborating literature values for the Log K_{ow}'s and the range of those values. These data were selected from the Pomona College Medicinal Chemistry database and the Log K_{ow}'s are the Log P star values from that database.

TABLE 1

Compounds Used in This Study

	Log K_{OW}	Range	Number of References
Phenol	1.46	1.46-1.75	12
2-Ethoxyphenol	1.68	1.68	1
Benzene	2.13	2.03-2.34	15
2-Nitrotoluene	2.30	2.30-2.36	2
Ethyl Benzoate	2.64	2.20-2.64	2
Chlorobenzene	2.84	2.46-2.89	6
Nitrobenzene	1.85	1.79-1.85	6
Anisole	2.11	1.98-2.11	5
Toluene	2.73	2.11-2.80	7
Biphenyl	4.09	3.76-4.17	8
Ethylbenzene	2.64	2.20-2.64	2
o-Xylene	3.12	2.77-3.12	2
Bromobenzene	2.99	2.98-3.01	3
1-Napthol	2.84	2.31-2.98	3

RESULTS AND DISCUSSION

Pump Stability

For accurate V_d and V_{ra} measurements, the pumping flow rate accuracy and precision were assessed. The aqueous flow rate was 4.890 mL/min with a standard deviation of 0.00579 mL/min (n=9) checked periodically over an 85-day period. The octanol flowrate was 0.317 mL/min with a standard deviation of 0.00217 (n=7) over a 55-day period. The flow rates were determined by timing volume displacement into volumetric flasks during the course of an analysis. Propagating this random variation into the K_{ow} results in only ±1.70% relative error at 95% confidence.

Selection of V_d

If the V_d selected is too small then the V_{ra} cannot be determined because the peak merely decreases from an initial maximum. For the same compound, as the V_d increases, the peak goes from a monotonicly decreasing profile to a Gaussian profile. If V_d is too large, then the later eluting portion of the peak appears truncated because some of the component eluted in the descending mode. An extreme of this situation is that the component completely elutes in the descending mode and is not even observable in the ascending mode.

Gaussian shaped peaks are not expected in chromatography if there are less than about 24 theoretical plates (8). A compound with a high partition coefficient for the chosen V_d would not have many theoretical exchanges between the phases and would thus not appear symmetrical. The peak maximum in these non-symmetrical peaks was not an accurate measure of V_{ra}. Ideally, the peak centroid should have been used. We found it difficult to assess the peak centroid because of the large error in determining the baseline of broad, unsymmetrical peaks. Figure 3 and Table 2 show the error in Log K_{ow} CPC versus the V_{ra}. At V_{ra}'s greater than 8 mL, the bias

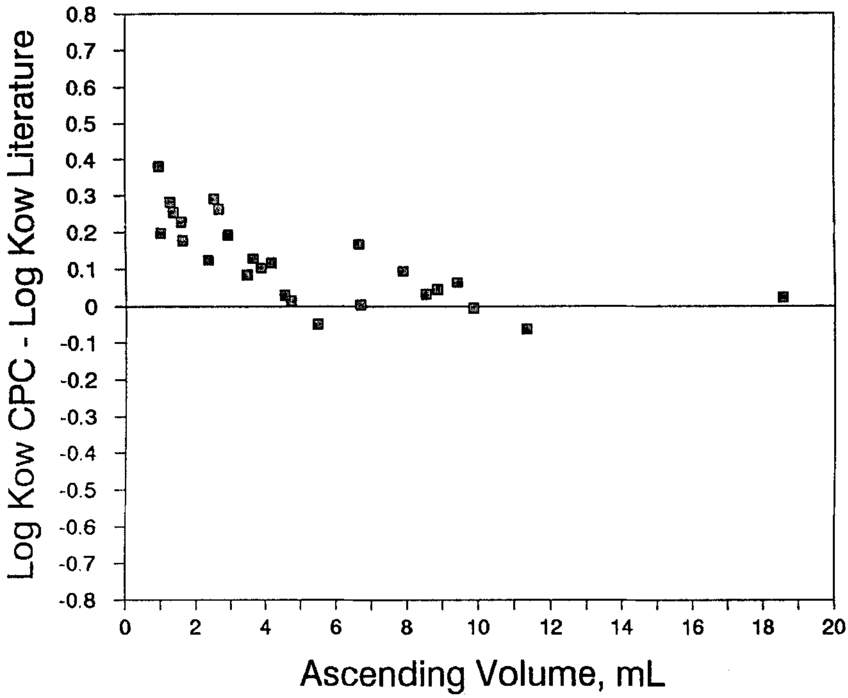

Figure 3. Error in Log K_{ow} Determined Versus Ascending Retention Volume, V_{ra}.

appears to be minimal relative to the random portion of the error. Thus, we estimate K_{ow}, either by a fragment method (9) or by a screening CPC run. We then select a V_d which will result in a V_{ra} large enough to minimize the bias in practical applications.

Results from Maximum V_d

Most papers which present alternative procedures for determining K_{ow} invariably show a plot of K_{ow} determined versus K_{ow} literature. In the case of this methodology, an analogy is to

TABLE 2

Compound	V_d	V_a	Log K_{ow}	Log K_{ow} Literature	Error
Phenol	77.27	1.581	1.69	1.46	0.22
Phenol	157.1	4.154	1.58	1.46	0.11
Phenol	313.5	9.394	1.52	1.46	0.05
2-Ethoxyphenol	235.3	2.506	1.97	1.68	-0.01
2-Ethoxyphenol	470.0	6.653	1.85	1.68	-0.13
Benzene	308.7	0.949	2.51	2.13	0.38
Benzene	616.7	2.922	2.32	2.13	0.19
Benzene	1,233	8.493	2.16	2.13	0.03
2-Nitrotoluene	489.6	1.276	2.58	2.30	0.26
2-Nitrotoluene	987.6	3.638	2.43	2.30	0.11
2-Nitrotoluene	1,957	7.883	2.39	2.30	0.07
Ethyl Benzoate	1,067	1.361	2.89	2.64	0.25
Ethyl Benzoate	2,133	2.659	2.90	2.64	0.26
Ethyl Benzoate	4,263	8.801	2.69	2.64	0.05
Chlorobenzene	1,688	1.615	3.02	2.84	0.18
Chlorobenzene	3,380	4.717	2.86	2.84	0.02
Nitrobenzene	342.9	4.525	1.88	1.85	0.03
Nitrobenzene	690.1	9.852	1.85	1.85	0.00
Nitrobenzene	1,389	20.48	1.83	1.85	-0.02
Anisole	631.4	3.863	2.21	2.11	0.10
Anisole	1.262	8.833	2.16	2.11	0.05
Anisole	2,524	18.55	2.13	2.11	0.02
Toluene	2,627	5.477	2.68	2.73	-0.05
Toluene	5,248	11.30	2.67	2.73	-0.06
Biphenyl	19,595	1.010	4.29	4.09	0.18
o-Xylene	4,103	2.333	3.25	3.12	0.13
Bromobenzene	4,103	3.464	3.07	2.99	0.08
1-Napthol	4,671	6.677	2.84	2.84	0.00

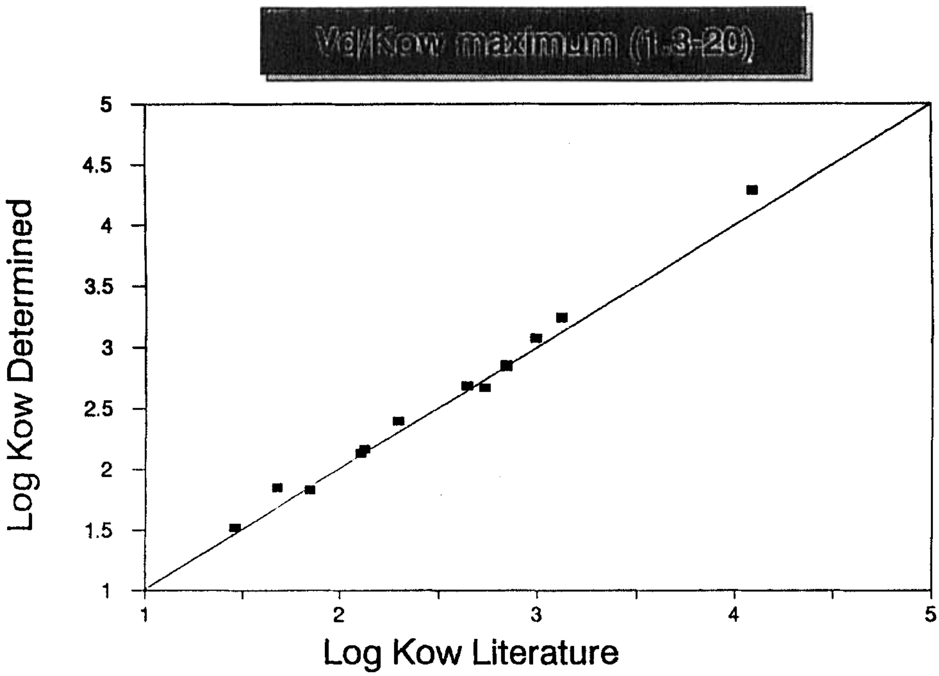

Figure 4. Log K_{ow} Determined Versus Log K_{ow} Literature for All Compounds at the Maximum V_d.

show (Figure 4) this plot taken for all the compounds used in this study at maximum V_d in the cases where several V_d's were chosen for the same compound. The determination range in this technique was up to Log K_{ow} literature of 4.09 (Kow=12,300) and the determined K_{ow} values agreed well with the literature values.

CONCLUSIONS

Dual-mode CPC was effective in determining K_{ow}'s up to 12,300. The Dual-mode CPC system complements the normal elution mode of

CPC for determining K_{ow}'s with a demonstrated range of 29 to 12,300 K_{ow} (1.5 to 4.1 log K_{ow}). The procedure was fundamentally derived without requiring the use of extra-thermodynamic parameters. The technique has a determination bias dependent on the distance the material traveled through the CPC system as reflected in the V_{ra}. For the best accuracy, the V_{ra} should be at least 8 mL. Below 8 mL, the bias could perhaps be subtracted and this would lead to even faster analysis times. For this reason, we are currently developing a theoretical basis for subtracting this bias.

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