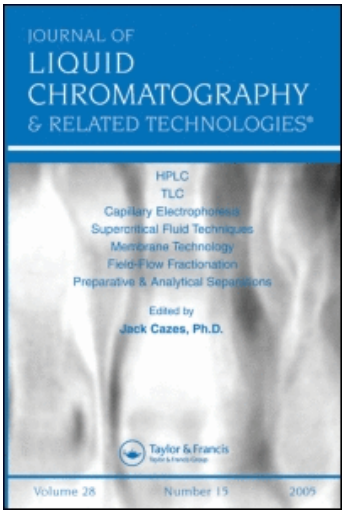

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S. J. Gluck[a]; E. J. Martin[b]
[a] The Dow Chemical Company Analytical Sciences Laboratory Building, Midland, Michigan [b] DowElanco 2800 Mitchell Drive Walnut Creek, California

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ASSESSMENT OF CENTRIFUGAL PARTITION CHROMATOGRAPHY FOR DETERMINATION OF OCTANOL-WATER PARTITION COEFFICIENTS

S. J. GLUCK[1] AND E. J. MARTIN[2]
[1]*The Dow Chemical Company*
Analytical Sciences Laboratory
Building 1897
Midland, Michigan 48667
[2]*DowElanco*
2800 Mitchell Drive
Walnut Creek, California 94598-0902

ABSTRACT

Centrifugal partition chromatography (CPC) has been assessed as a convenient automated method for the determination of octanol-water partition coefficients (K_{ow}) over the range of -0.5 to 2.5 log units. The stationary (V_s) and mobile phase (V_m) volumes, which are needed for the calculation of K_{ow}, are determined in situ by injecting four compounds with known K_{ow}. V_s and V_m were also determined by independent analytical means to demonstrate that this is a direct measurement of K_{ow} from fundamental chromatographic principles with no unexplained fitted parameters. Propagation of error shows that a single four-component calibration with duplicate injections of each unknown is sufficient to determine log K_{ow} with a precision of less than 0.1 log units.

INTRODUCTION

Octanol-water partition coefficients (K_{ow}) have been established as the most significant quantitative physical property correlated with biological activity (1). In this capacity, they

have found extensive use in drug and pesticide design as a parameter for quantitative structure activity relationships (QSAR). In addition, log K_{ow} is used to predict bioconcentration factors in aquatic organisms, water solubility and soil adsorption coefficients (2). Because of these relationships between K_{ow} and environmental parameters, state and federal agencies in the United States require the accurate determination of K_{ow} by prescribed methodologies for product registration (3). These procedures, which are commonly referred to as the "shake-flask" and "generator-column" methods, are sensitive to impurities and, thus, require a selective detection scheme such as HPLC. They are expensive to practice due to the effort required to assure an accurate measurement and the development of HPLC procedures for each compound. They are not amenable to automation for a set of unrelated compounds. Hence, use of the accurate government approved methodology is not practical for routine K_{ow} determination for QSAR purposes.

Alternative means have, therefore, been developed for estimating K_{ow}. Additive molecular fragment approaches have been developed which have the advantage that they can be used on compounds for which no sample is available (2, 4, 5). Extra-thermodynamic relationships have also been found which correlate log K_{ow} with the log of reversed phase liquid chromatography capacity factors using empirically fitted but theoretically unexplained constants (6, 7). (There are over 100 publications which attempt to correlate HPLC capacity factors to K_{ow}.) Besides ease of automation, HPLC has the important advantage of insensitivity to impurities and small sample requirements. Both of these approaches work best for estimating K_{ow} of structural homologs, however, and work poorly for structurally unrelated compounds (6-11). Thus, they are generally not approved by regulatory agencies. In addition, the HPLC procedure is limited to a pH range under which the packing material is stable, and thus is poorly suited for moderately strong acids and bases. In general, these approaches do not have the accuracy desired for

quantitative work because they only mimic true octanol-water partitioning. They are not direct measurements of K_{ow}.

Centrifugal partition chromatography (CPC) using octanol and water as the two phases has been shown to be feasible for providing octanol-water partition coefficients (12-14). This approach is attractive because the retention mechanism is the partitioning of a solute between octanol and water. It offers the automation advantages, small sample size, and insensitivity to impurities of the HPLC procedures with the potential accuracy of the shake-flask method. The mere fact that octanol and water are the chromatographic phases does not, however, guarantee a direct measurement. The system must be proven to be in equilibrium and the fundamental chromatographic relationships on which the partition coefficient calculations are based must be shown to hold. In a feasibility study, Berthod and Armstrong used CPC to determine K_{ow} for a set of 17 structurally diverse compounds (14). The method was stated to be direct because K_{ow} could be correctly determined from the retention volume (V_r) and the stationary phase (V_s) and mobile phase (V_m) volumes on the basis of chromatographic theory. However, the method by which they determined V_s, which is needed to deduce K_{ow} from V_r, implicitly assumed the theory which they wished to demonstrate. Any extra-thermodynamic linear relationship between K_{ow} and V_r would have given the appearance of a direct measurement, except that "V_s" would have been a theoretically unexplained fitted constant with a value different from the actual stationary phase volume. In that case, applying the method to diverse compounds outside of the training set would be far less secure. This work verifies the assumptions in Armstrong and Berthod's theory, demonstrating that CPC is not just another empirical estimation method but is in fact a direct measurement of K_{ow}. Being direct, it can be expected to give accurate K_{ow} determinations for structurally diverse compounds.

The precision of the procedure has been stated to be dependent on the V_r of the compound (14). It is useful to ascertain what procedures are required to obtain a desired

precision. Replicate runs of a sample do not suffice because the result is also dependent on the precision of the determination of system parameters such as V_s and V_m. A propagation of errors analysis is presented to determine precisions over the range of application.

EXPERIMENTAL

Apparatus

The system consisted of a CPC, Model CPC-LLN, Sanki Laboratories, Sharon Hill, PA. A CPC is a liquid-liquid chromatographic apparatus wherein the stationary phase remains in place due to a centrifugal force (12, 15, 16). Mobile phase flowed from a 10 liter reservoir through the Sanki Laboratories Model LBP-V pump into a Hitachi Model 655A-40 autosampler. Analyte partitioning took place in the centrifugal rotor, which was thermostated at 20°C. Six rotor cartridges were used, corresponding to 2400 individual extraction stages of approximately 50 µL each. Compounds were detected with a Kratos Model 757 variable wavelength UV absorbance detector equipped with a preparatory, short pathlength, flowcell. The UV detector was followed with a Molytek Thermalpulse flow rate monitor. The absorbance and the flow rate outputs were monitored with the PE Nelson ACCESS*CHROM chromatography datasystem (Figure 1).

Filling the Cartridges with Stationary Phase

Isopropanol was used to flush the system before 150 to 250 mL of water saturated octanol was pumped through the system to completely fill the cartridges with octanol. While the rotor was spinning at 300 rpm, octanol saturated water was pumped in the reverse-ascending mode as fast as possible without over-pressurizing the system until the first drop of water appeared at the detector exit as indicated by both the appearance of an emulsion and a large shift in the absorbance output. Pumping in

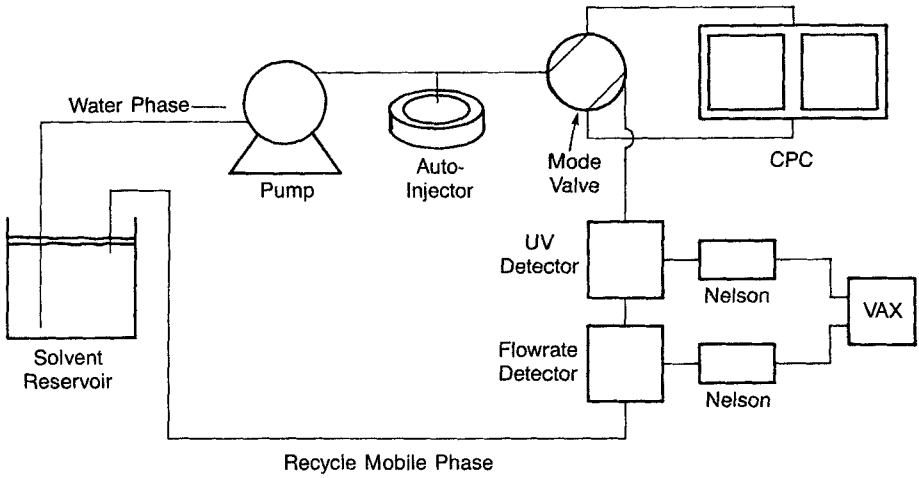

FIGURE 1

System Block Diagram

this direction is supposed to empty the system of stationary phase but in practice some remains, thus providing a smaller fraction of stationary phase and correspondingly shorter retention times. This operation has been referred to as "underloading" (14). The mode valve was then switched to the reverse-descending position and the water flow continued for 20 to 30 minutes at 8 to 20 mL/min. Under these high flow rates and low rpm conditions, more of the stationary phase is removed. Finally, the rotor speed was increased to 700 rpm and the flow rate was decreased to 4 mL/min. The baseline would usually stabilize within 20 minutes. Typical system pressure was 20 Kg/cm^2. Because the system was run in a recycle mode, it was necessary to place the detector effluent line near the top of the mobile phase reservoir and the pump inlet line was placed at the reservoir bottom.

For safety concerns and convenience, solvents were presaturated in a hood using a 3-L mixing apparatus. This

consisted of a round bottom, 3-neck, 3-L flask with a paddle-type electric stirrer and a Teflon stopcock affixed to the bottom of the flask. This apparatus minimized the handling of solvents and assured well saturated phases.

Retention Volume Determination

The V_r of an analyte was determined by its retention time and either the integrated flow rate of the eluent with respect to time or the average flow rate multiplied by the time. Extensive comparisons between both procedures showed that the simple method of multiplying the average flow rate by retention time gave better reproducibility than integration of the flowmeter output in the retention volume measurements. The flowmeter was not well suited to the measurement of saturated solutions.

Analyte retention time was determined manually as the peak maximum using the post-run plotting capabilities of the PE Nelson ACCESS*CHROM software on a VAX-based datasystem. Data were collected at a 1-point-per-second rate. Run times varied from 25 minutes to 16 hours, depending on the K_{ow} of the analyte.

Standards and Analytes

The standard mixture consisted of 5% phenol, 8% benzyl alcohol, 1% benzamide and 2% dimethylformamide in octanol. All of these compounds can be detected at 255 nm. The analytes consisted of a mixture of acetone, acetanilide, acetophenone and 2-chlorophenol in octanol. These compounds could all be detected at 227 nm. Repetitive 100 µL injections of the standards were performed by the autosampler. Data collection was started at the time of injection by the autosampler. In this mode, the system can run for weeks without maintenance. Literature values for all compounds were selected as Log P Star values from the Pomona College Medicinal Chemistry database (17). Typical sample masses injected ranged from 0.5 to 5 mg.

Effect of Flow Rate

Determining the effects of flow rate required a precise measurement of flow, which was done by determining with the ACCESS*CHROM software the time required to fill various size volumetric flasks. The experiment was performed by injecting standard mixtures at 7.578, 5.786, 4.050, 1.763 and 0.4918 mL/min in the order as listed.

Analytical Measurements of Phase Volumes

The total system volume (V_t) was measured by injecting 100 µL of 10% dimethylformamide into the system containing only isopropanol, with no stationary phase, at a premeasured flow rate. From the retention time of the dimethylformamide (255 nm) and the known flow rate, V_t was determined to be 119.4 mL with an estimated precision of ± 1.2 mL.

The mobile phase volume, V_m, and stationary phase volume, V_s, were determined by disconnecting the rotor before the autosampler and applying 200 psig nitrogen to empty the cells. This action resulted in the collection of 106 mL of mobile phase. The system was then flushed thoroughly with isopropanol. The volumes of octanol and water in the isopropanol were determined by gas chromatography. Corrections were made for a 3.20 M (0.04 v/v) solubility of water in octanol (18) and a 4.5 mL volume of tubing before the injector and after the detector. The V_m was 105.5 mL ± 2, and the V_s was 13.3 ± 1.3. The sum of these two volumes, 118.8 mL with an estimated precision of ± 2.4 mL, equals V_t and agrees with the value for V_t determined above. The values for V_m and V_s were also calculated from the retention volumes of known compounds. This is described in the CALCULATIONS and the RESULTS sections.

CALCULATIONS

The determination of K_{ow} from V_r is based on the fundamental chromatographic relationship:

(1) $$V_r = V_s * P + V_m$$

where P is the partition coefficient (12-14). If octanol is the stationary phase and water is the mobile phase, then P is K_{ow}. Knowing the system parameters V_m and V_s, K_{ow} is obtained as a linear function of the only compound dependent variable, V_r:

(2) $$K_{ow} = (V_r - V_m)/V_s$$

Several approaches have been reported for determining V_m and V_s. Terada (19) determines V_m by measuring the amount of stationary phase displaced by mobile phase into a graduated cylinder during start-up. The V_s is then calculated from the difference between V_m and the independently known instrument constant V_t:

(3) $$V_s = V_t - V_m$$

P is then calculated from eq. 1 (Terada did not use the octanol-water solvent system).

Berthod and Armstrong (13, 14) determined V_s and V_m from V_r for a standard compound with a known K_{ow}, using equations 2 and 3 along with the independently determined value for V_t.

Our approach is to determine V_s and V_m from a set of 4 compounds of known K_{ow} covering a wide range of values. From Equation 1, the slope of a plot of V_r versus literature K_{ow} is V_s, and the intercept is V_m. Since the uncertainty in V_r increases with increasing K_{ow}, weighted regression is used to determine the slope and intercept (20). The weights are reciprocal variances from 33 identical injections of the calibration mixture (see RESULTS). System calibration may be repeated several times for improved accuracy. After establishing V_s and V_m, equation 2 is used again to determine K_{ow} of the unknowns. Since equation 3 is not used in our procedure, it serves as an internal check for consistency in V_m and V_s. Futhermore, separate experiments

described below show that this slope and intercept correspond with independently determined values for V_s and V_m.

The precision of the K_{ow} determination for an unknown compound can be assessed by analyzing the propagation of random error. The uncertainty in determining log K_{ow} from Equation 2 is given by (21):

$$\lambda(\log K_{ow}) = 0.4343 * \sqrt{\frac{(\lambda^2 V_r + \lambda^2 V_m)}{(V_r - V_m)^2} + (\lambda V_s / V_s)^2} \quad (4)$$

Where λ indicates the 95% confidence interval.

Estimates for the standard deviations in V_s (sV_s) and V_m (sV_m) are calculated from the standard deviations of the slope and intercept from the weighted regressions of the 33 identical injections of the standard mixture described above. $\lambda(V_m)$ and $\lambda(V_s)$ for a new experiment are then calculated from those standard deviations along with t for the total number of observations in the calibration runs for the current experiment minus 2. (Two degrees of freedom are required to determine a line).

$$\lambda(V_m) = t*sV_m \quad (5)$$

$$\lambda(V_s) = t*sV_s \quad (6)$$

The 95% confidence interval for the mean value of V_r ($\lambda(V_r)$) is calculated from the standard error of the mean for V_r of the compound to be tested:

$$\lambda(V_r) = (t*sV_r)/\sqrt{n} \quad (7)$$

where n is the number of replicate injections of the test sample, and t is for 95% confidence at n-1 degrees of freedom. This requires an estimate for the standard deviation of V_r (sV_r) for

the unknown. An empirical estimate of $s(V_r)$ as a function of K_{ow} is described below (see RESULTS).

RESULTS AND DISCUSSION

This apparatus and procedure were designed for the unattended, automatic measurement of standards and samples. Hence, the eluent is recycled and a data system has been employed to collect raw data for manual post-run manipulations. Ideally, the data system could determine the V_r of each compound automatically, but the high viscosity of the octanol stationary phase resulted in broad peaks for the more lipophilic compounds. This low efficiency leads to poor signal-to-noise ratios for these compounds and the chromatography data algorithms have trouble picking the peak maximum. In addition, small amounts of stationary phase occasionally collect slowly and release rapidly in the detector flowcell resulting in significant baseline shifts. These shifts, combined with wide peaks and occasional low signal-to-noise ratios mandate a manual determination of V_r.

System Calibration and Characterization

The system's precision was studied by injecting the set of four calibration compounds 33 consecutive times, once every 334 minutes. A typical chromatogram is shown in Figure 2. The V_r's are in Table I. The V_s was determined for each individual injection by the weighted regression procedure (see below), and plotted versus the cumulative volume pumped (Figure 3). The slope of this line shows the volume of stationary phase increased by 0.6 mL (2.6%) over the course of the week long experiment. This very slight increase of 3.3 μL/hr is actually less than the expected increase of 15 μL/hr due to 84 μL of octanol added with each injection. This indicates a slow bleed rate of 12 μL/hr which is only 7% of that in a previous report (12). This difference might be due to a lower operating pressure or better octanol pre-saturation of the mobile phase from recycling the

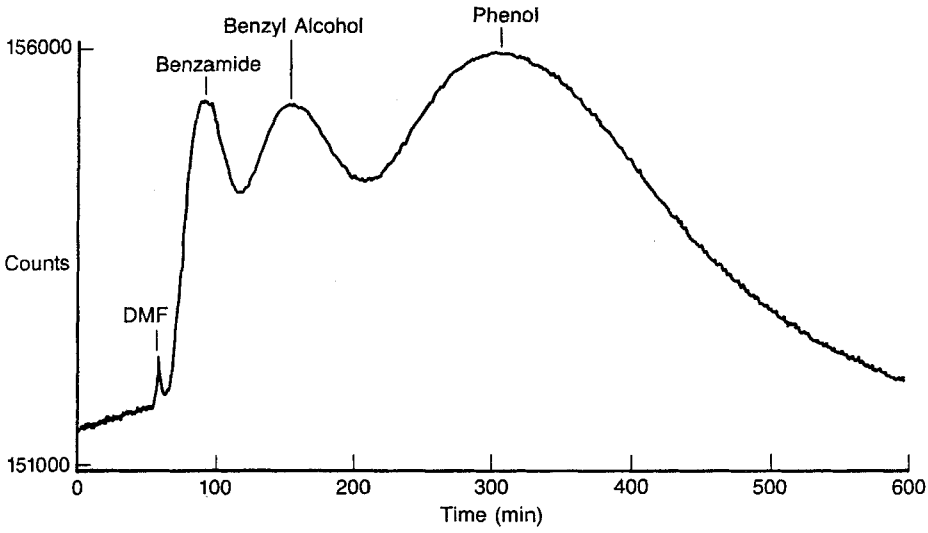

FIGURE 2

Typical Chromatogram of the Calibration Mixture

solvent. Since the autoinjector reruns the standard mixture every 2 or 3 days, these small volume changes can easily be monitored and the instrument can be operated for weeks without recharging the centrifuge. This is an important reason for determining V_s and V_m *in situ* by the weighted regression procedure rather than using analytical methods when the instrument is loaded or unloaded.

Weighted Least Squares Regression

A plot of V_r versus K_{ow} for the combined data from 33 injections of the calibration set (Figure 4) shows the basic linear relationship of Equation 1. The scatter in V_r increases with K_{ow}. Since ordinary least squares regression assumes that errors in the y-values are constant, this variation of sV_r with K_{ow} dictates the use of weighted regression.

TABLE 1

Retention volumes (mL) for 33 replicate injections of a standard mixture. Values not determined were due to occasional signal-to-noise problems inherent in trying to use one wavelength to monitor these four compounds.

Run #	Dimethylformamide	Benzamide	Benzylalcohol	Phenol
1	*	194	369	799
2	*	193	366	774
3	*	*	367	760
4	97.5	194	370	791
5	98.4	195	372	795
6	97.3	193	362	*
7	*	192	363	783
8	97.5	193	369	782
9	97.8	194	363	772
10	97.4	194	366	771
11	•	194	369	794
12	97.6	194	364	*
13	*	193	379	809
14	*	195	374	789
15	97.3	193	369	764
16	*	193	362	*
17	*	195	371	792
18	*	195	373	787
19	97.0	193	371	786
20	97.3	194	366	771
21	97.0	194	363	785
22	96.8	194	369	804
23	97.1	195	371	785
24	96.9	193	372	789
25	96.8	194	373	777
26	96.7	198	373	811
27	*	199	377	803
28	*	194	372	788
29	*	193	376	783
30	96.6	195	375	824
31	96.8	196	378	*
32	*	195	369	779
33	96.4	194	368	794
Mean	97.2	194.	370.	788
Std.dev.	0.5	1.4	4.6	14.3
Literature K_{ow}	0.0977	4.36	12.6	29.5

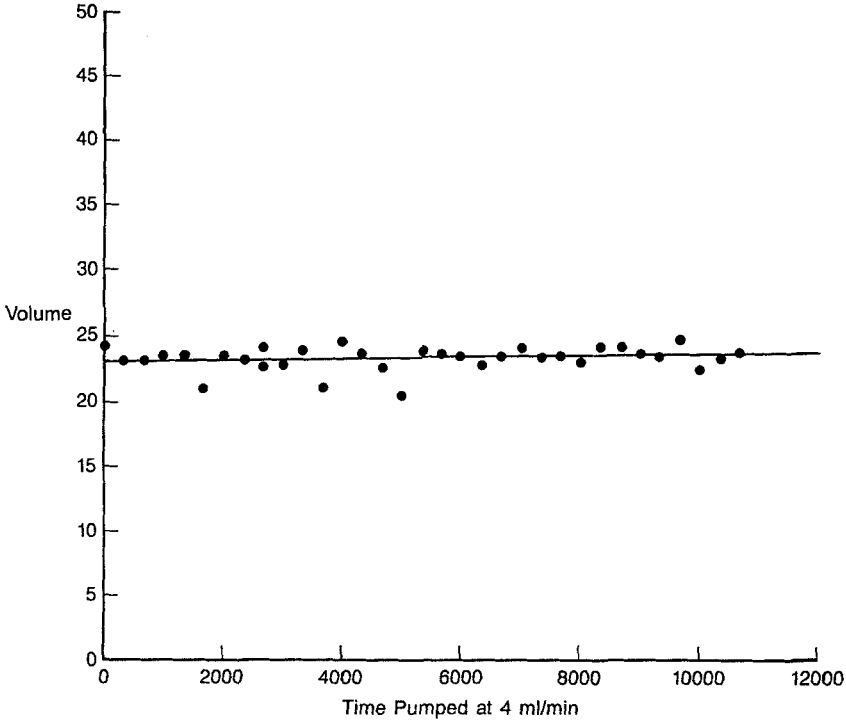

FIGURE 3

Stationary Phase Volume Versus Time Pumped at 4 mL/min
Slope = $5.5 \cdot 10^{-5}$ mL/min, Intercept = 23.1 mL

Table II compares the calculated phase volumes from ordinary and weighted regression. The total volume ($V_t = V_s + V_m$) from non-weighted regression is far below the actual system volume of 119 mL ± 1.5 mL. Also, the uncertainty in V_m from ordinary regression is over ten-fold greater than from weighted least squares. These errors would be reflected in the computed K_{ow}.

Test Samples

Four compounds: acetone, acetanilide, acetophenone and 2-chlorophenol, were run as a set of unknowns. Before these

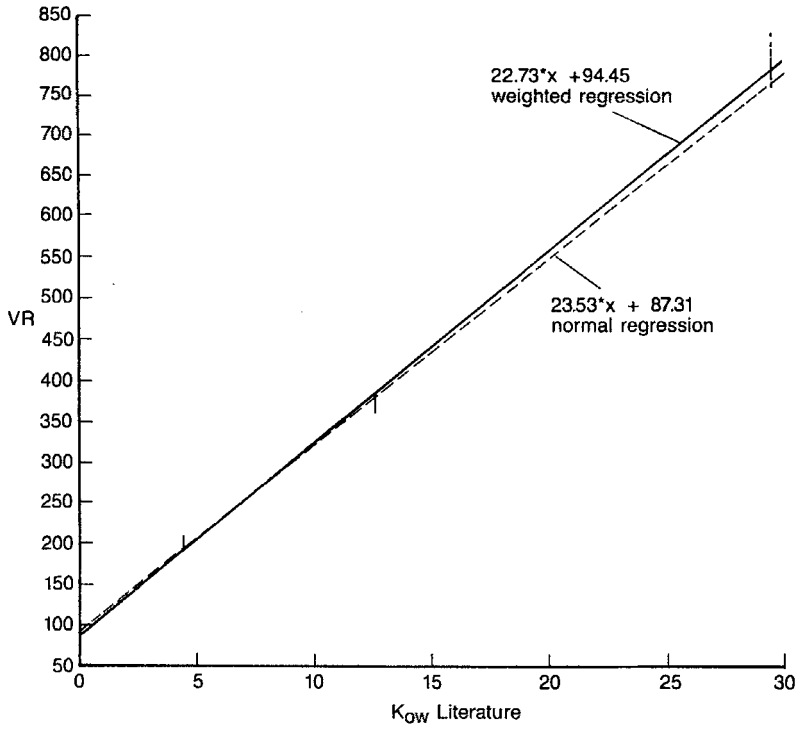

FIGURE 4

Retention Volume Versus Log K_{OW} for 33 Injections of the Calibration Mixture

TABLE 2

Comparison of Ordinary and Weighted Regression for 33 Injections of the Calibration Mixture Error Represents 95% Confidence Interval

	Weighted Linear Regression	Linear Regression
Mobile Phase Volume (mL)	95.00 ± 0.25	87.31 ± 3.35
Stationary Phase Volume (mL)	22.60 ± 0.15	23.53 ± 0.20
Total Phase Volume	117.6 ± 0.29	110.8 ± 3.4
R-squared	0.999	0.998

TABLE 3

Test Compounds Determined by CPC Using the Weighted Regression Procedure

The V_s was 20.24 ± 0.40 mL, V_m was 97.57 ± 0.20 mL, and V_t was 117.8 ± 0.45 mL, as Determined by Four Injections of the Calibration Mixture.

	Literature		CPC determined	
Compound	log K_{ow}	V_r (mL)	log K_{ow}	Residual
Acetone	-0.24	107.0	-0.31	0.07
Acetanilide	1.16	394.5	1.16	0.00
Acetophenone	1.58	890.8	1.59	0.01
2-Chlorophenol	2.15	2716	2.11	0.04

compounds were injected, the system was calibrated by four injections of the standard mixture. Table III contains V_r, literature log K_{ow}, and CPC log K_{ow} for each test compound. All the CPC K_{ow} values agree well within the targeted ± 0.1 log units of the literature K_{ow} values.

Comparison to Other Calculation Procedures

Table IV shows the literature K_{ow}'s for the four test compounds compared to values obtained using quadruplicate injections of each of 4 separate compounds in single point determinations of the stationary phase volume as in the procedure of Berthod and Armstrong (13, 14). Comparing the results to the four-point procedure (last column) illustrates the improved accuracy of the weighted least squares regression. Furthermore, the results from the single compound method showed considerable variation depending on the specific choice of calibration compound.

Demonstration of a Direct Method

The validation of this procedure as a direct determination of K_{ow}, *i.e.*, one with no theoretically unexplained fitted

TABLE 4

Log K_{ow} Measurements for 4 Test Compounds Comparing Four Component Weighted Regression With the Single Component Calibration Method of Berthod and Armstrong

		Berthod and Armstrong				
Compound	log K_{ow} Literature	Dimethyl-formamide V_s=21.99 mL	Benzamide V_s=19.63	Benzyl-alcohol V_s=19.29	Phenol V_s=21.08	4-component Weighted Regression
Acetone	-0.24	-0.36	-0.44	-0.45	-0.39	-0.31
Acetanilide	1.16	1.13	1.18	1.18	1.15	1.16
Acetophenone	1.58	1.56	1.61	1.61	1.58	1.59
2-Chlorophenol	2.15	2.08	2.12	2.13	2.09	2.11

parameters, is a key aspect of this study. Indirect procedures such as HPLC, Terada's CPC method, and Tayar's CPC method (22), which rely on empirical correlations between octanol-water partitioning and other partitioning phenomena can yield erroneous results, particularly when applied to structurally diverse compounds.

The fundamental relationship (Equation 1) assumes that the system is in equilibrium. If the system were poorly mixed, short-circuit fluid flow or channeling could occur (23), which would decrease the effective V_m and/or V_s. Increased mixing or lower flow rates would then be expected to increase V_r. The calibration set was run at 700 rpm from 0.4918 to 7.578 mL/min and was used to examine the impact of flow rate. The resulting chromatograms, given in Figure 5 with the volume axes normalized to 800 mL, show that the slower the flow rate, the more efficient the separation over this range. Table V, which lists the V_s, V_m and V_t calculated from weighted least squares regression of measured V_r values, however, shows that flow rate has no effect on V_r or the system parameters used to determine K_{ow}. Furthermore, the instrument was emptied and V_s, V_m, and V_t were directly measured immediately after this experiment (see EXPERIMENTAL).

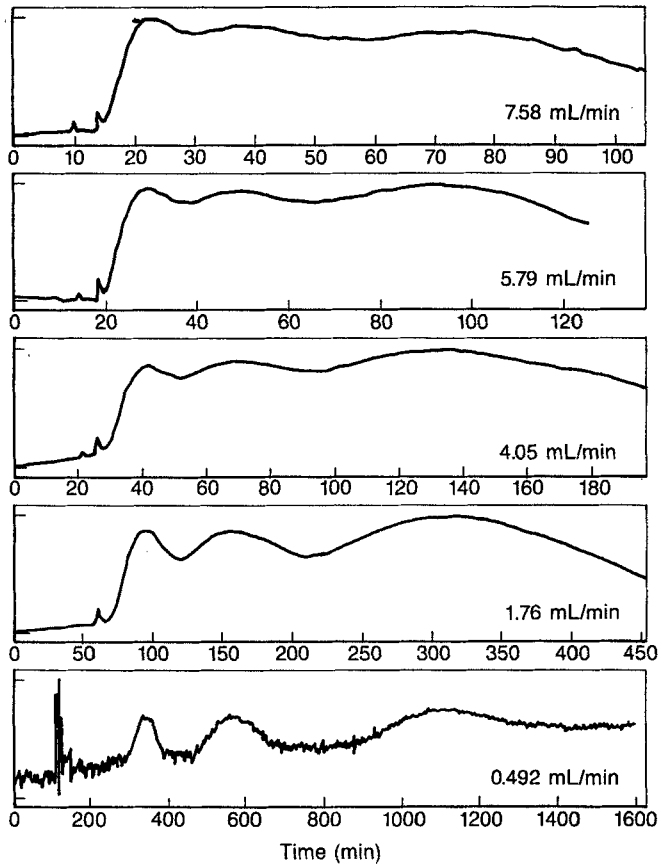

FIGURE 5

Effect of Flow Rate on Retention Volume and Efficiency
Volume Axis Equals 800 mL

TABLE 5

V_s, V_m and V_t Determined by Weighted Least Squares of V_r's at Different Flow Rates, with 95% Confidence

Flow Rate (mL/min)	V_m (mL)	V_s (mL)	V_t (mL)
7.578	102.9 ± 0.72	15.1 ± 0.83	118.0 ± 1.2
5.786	104.1 ± 0.33	14.3 ± 0.43	118.4 ± 0.54
4.050	103.4 ± 0.83	14.5 ± 1.17	117.9 ± 1.43
1.763	105.4 ± 0.94	14.2 ± 1.6	119.6 ± 1.9
0.4918	102.4 ± 0.86	14.4 ± 0.68	116.8 ± 1.1
All flow data combined	104.2 ± 0.52	14.4 ± 0.65	118.6 ± 0.83

TABLE 6

V_r's (mL) for Four Compounds Versus Rotor Revolutions Per Minute

	300 rpm	800 rpm	1300 rpm
DMF V_r	92.84	93.08	93.75
	95.79	92.95	93.66
Benzamide V_r	192.8	188.3	188.9
	187.6	188.8	188.1
Benzyl alcohol V_r	369.5	366.6	363.0
	370.7	369.6	363.0
Phenol V_r	777.2	780.1	788.8
	782.8	787.4	785.3

The directly measured values for V_m and V_s of 105.5 ± 2 mL and 13.3 ± 1.3 mL agree with the values in Table V for each flow rate, as well as the average over all flow rates.

The impact of centrifugal force on mixing and equilibrium was determined by replicated injections of the standard mixture at 300, 800 and 1300 rpm (4.5 mL/min). The data are presented in Table VI. Since pairs of values would not give very accurate standard deviations, the error analysis was performed using standard deviations in V_r from a previously described experiment of 33 identical injections of these same compounds (Table I). Both t-tests and analysis of variance show that the V_r's do not change with spinning rate, as expected for a system in equilibrium. No change in separation efficiency was observed either, in potential contrast to previous observations using other phases (22, 24).

Since we are operating in underload mode, V_s and V_m are under experimental control and may be varied over a wide range each time the CPC is charged with octanol. Their sum, V_t, however, is a geometric parameter and should remain constant as long as the hardware is not changed. Tables II, III and V show V_s, V_m and V_t from three different loadings of the instrument throughout the course of this study. Although V_s and V_m vary, their sum, V_t,

always agrees, within experimental error, with the values of 119.4 and 118.8 determined by the two independent procedures described in the EXPERIMENTAL section.

Together, these experiments demonstrate that the system is in equilibrium throughout the practical range of operating conditions. Furthermore, the slope and intercept of the calibration line relating V_r to K_{ow} do indeed correspond to the physical system parameters described in Equation 1, as determined by independent analytical means. Thus, we conclude that the procedure we describe is a direct measurement of K_{ow}.

Propagation of Errors

A goal of this project was to develop a CPC procedure to determine log K_{ow} ± 0.1 at 95% confidence throughout the range of 0 to +2.5. Values below zero are best measured in normal-ascending mode (with octanol as the mobile phase) and might be expected to have uncertainties comparable to those of their positive counterparts. Propagation-of- error analysis helps to clarify the experimental protocol required to achieve this precision. The relevant equations (Equations 4-7) were presented above in the CALCULATIONS section. With these equations, the expected uncertainty in measured K_{ow} can be calculated as a function of actual K_{ow} throughout the desired range.

Experimental values for sV_m, sV_s, and sV_r (as a function of V_r) are required to solve Equations 5-7. From the weighted regressions for 33 replicate injections of the standard mixture, the standard deviation of the slope and intercept (sV_s and sV_m) were found to be 0.098 and 0.099, respectively. Plotting sV_r versus the mean of V_r for each of the four calibration standards revealed a simple linear correlation.

(8) $$sV_r = 0.0206*V_r - 2.295$$

$$n = 4 \quad r^2 = 0.988 \quad s = 0.836 \quad F = 169.7 \quad (p=0.004)$$

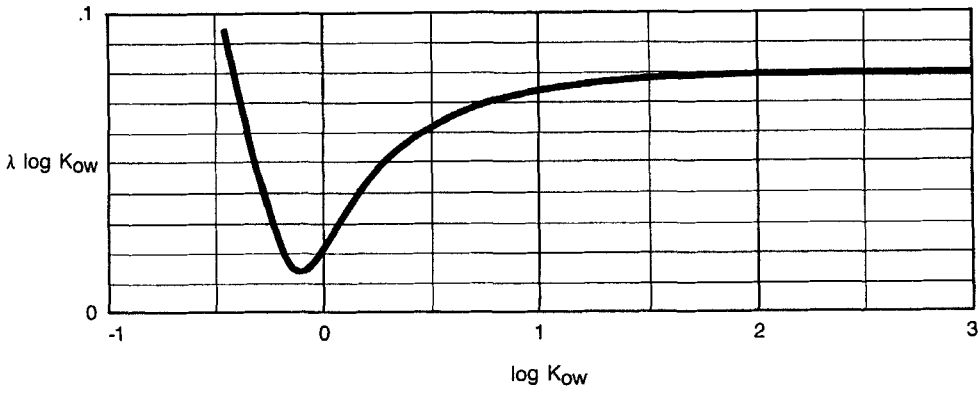

FIGURE 6

Error in Log K_{ow} Versus Log K_{ow} at 95% Confidence

Thus Equations 5-8 can be used to calculate the expected random experimental error in a CPC log K_{ow} determination as a function of actual log K_{ow} of an unknown. Examination of Equation 4 shows that for compounds with very low K_{ow}, which are barely retained so $V_r - V_m$ is small, the relative uncertainty in K_{ow} will be large. This limits the low range of K_{ow} that can be measured with water as the mobile phase. Equations 7 and 8 show that sV_r, and therefore $\lambda(V_r)$, increase linearly with V_r (and therefore K_{ow}). Thus, both the numerator and the denominator of Equation 4 increase at the same rate when V_r is much larger than V_m, and the relative uncertainty in K_{ow} approaches a constant value for lipophilic compounds. There is a minimum at intermediate K_{ow} values where $\lambda(V_r)$ is small but V_r is still substantially greater than the dead volume, V_m. All uncertainties depend on the t values and, therefore, on the number of replications of each part of the experiment. Figure 6 shows the expected error in CPC-determined log K_{ow}, as a function of actual log K_{ow}, for an experiment with a single injection of the four-compound calibration mixture and duplicate injections of each unknown.

This procedure is seen to be sufficient to achieve the desired precision throughout the log K_{ow} range of -0.5 to 2.5.

CONCLUSIONS

An automated CPC technique has been developed for determining log K_{ow} in the range from -0.5 to 2.5. In contrast to previous chromatographic methods (6-11), the procedure was shown to be a direct measurement of K_{ow} with no empirically fitted parameters. A multi-point *in situ* calibration employing weighted regression was found more convenient and accurate than previous methods for determining the phase volumes (13, 14, 19), which is critical for accurate K_{ow} determination. Propagation of error showed that a single four-component calibration, followed by duplicate injections of the unknown provides, at 95% confidence, uncertainty of less than 0.1 log K_{ow} units over the accessible range.

The main practical shortcoming of this procedure is the limitation to log K_{ow} values less than 2.5. A cooperative effort with Armstrong et. al. (12-14, 16) to extend the range of the method is currently underway.

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