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ACKNOWLEDGMENTS

A portion of this work was presented at the 14th Central Regional Meeting of The American Chemical Society, Midland, Michigan, June 1982. This investigation was funded by research grants from the Epilepsy Foundation of America, The Upjohn Company, the University of Toledo Faculty Research Awards and Fellowships Program, and the Medical Research Council of Canada. The authors thank David L. Miskell and Eugen Kurylo for their technical assistance.

Accurate, Wide-Range, Automated, High-Performance Liquid Chromatographic Method for the Estimation of Octanol/Water Partition Coefficients I: Effect of Chromatographic Conditions and Procedure Variables on Accuracy and Reproducibility of the Method

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Received March 7, 1983, from the *Department of Animal Science, University of Illinois, 126 Animal Sciences Laboratory, 1207 West Gregory Drive, Urbana, IL 61801.* Accepted for publication November 29, 1983.

Abstract □ A high-performance liquid-chromatographic (HPLC) procedure is reported for estimation of the logarithm of the octanol/water partition coefficient, log P(o/w). This automated log P(o/w) measurement (ALPM) circumvents many inherent difficulties with the shake-flask method, yet gives high reproducibility and excellent overall correlation with shake-flask results. Partition coefficients for numerous structurally diverse chemicals, ranging from ~0 to ~8 log P(o/w) units, can be determined; however, values for zwitterionic compounds cannot be obtained. Additional advantages of ALPM include lower cost and greater safety when compared with other HPLC or shake-flask procedures. Chromatographic conditions (*i.e.*, flow rate and temperature) and variables (*i.e*, column length and solvent composition) affecting this method are discussed in detail. ALPM may also find application in quality control of HPLC columns, qualitative-quantitative analysis, and in computer-controlled method development and analysis.

Keyphrases ◻ HPLC-octanol/water partition coefficients □ Partition coefficients-octanol/water, HPLC

The logarithm of the octanol/water partition coefficient of a compound, log P(o/w), often parallels the biological effects of that substance (1, 2). Although log P(o/w) correlations are commonly used to optimize a specific biological response, they can also be a valuable predictor of adverse effects from chemical agents (3, 4). Consideration of this parameter in structure-toxicity as well as structure-activity studies might substantially reduce drug development costs (1). Furthermore, the increasing cost of animals and animal care, combined with growing public discontent over the use of animals in scientific research, will inevitably make prediction of toxicological responses much more important (5, 6).

Ideally, determination of the partition coefficient, P(o/w), requires measurement of the equilibrium ratio of the concentrations of a single component, X, dissolved in nonpolar and polar layers using simple separatory funnel shake-flask procedures (2):

$$P(o/w) = [X]_{octanol}/[X]_{water} \qquad \text{(Eq. 1)}$$

Although the polar phase is nearly always water, the choice of nonpolar phase is arbitrary; chloroform, hexane, and other solvents have been used, but octanol is the most common choice (2). There is, however, nothing unique about biological correlations using octanol/water as opposed to another solvent/water system. In 1954, Collander showed that partition data can be converted between solvent/water systems by least-squares regression (7), but Leo has described limitations in the use of this relationship (8).

Many economic and scientific problems occur with shake-flask log P(o/w) measurements. Since analytical procedures differ with each compound, the traditional method is time consuming and expensive (3). Log P(o/w) measurements may have only limited reproducibility among laboratories. For example, the seven reported shake-flask log P(o/w) values for naphthalene range from 3.01 to 3.59 (2). Analysis error is always a concern, but microemulsions can also alter distribution of the compound between the two phases (9). Formation of other components in the shake-flask could also alter phase equilibrium. The physical difficulties of measurement may be minimized by improved analytical techniques and sample centrifugation, but chemical interactions (changing the number of components) could affect the accuracy of even reproducibly obtained partition coefficients.

While measurement is always preferred, approximate values can be obtained by calculations based on the concept that the overall log P(o/w) reflects the summation of hydrophobic contributions from each constitutive group (π-approach) or fragment (f-approach) (2, 10, 11). Although calculated values often afford good estimates of log P(o/w), additivity of such constants may not always be observed (3). Problems of reproducibly and accurately measuring the shake-flask log P(o/w), knowledge of the increment for a particular molecular unit, and limitations in the additivity of such groups often re-

0022-3549/84/1100-1616$01.00/0

strict correlation analysis to academic research or the most rewarding industrial compounds.

There have been numerous attempts to employ high-performance liquid chromatography (HPLC) to estimate log P(o/w). Mirrlees *et al.* described the theory behind these efforts, most of which utilize the linear relationship between log P(o/w) and the logarithm of the capacity factor, log k', where $k' = [(t - t_0/t_0)]$ and t and t_0 refer to the elution times of compound and void volume marker, respectively (12):

$$\log P(o/w) = (m \times \log k') + b \qquad \text{(Eq. 2)}$$

Henry *et al.* modified Eq. 2 so that higher log P(o/w) values could be determined by defining log V_r, the logarithm of the retention volume, as a function of the flow rate, FR, according to (13):

$$\log V_r = \log [(t - t_0) \times FR] \qquad \text{(Eq. 3)}$$

They argued that since $\log k' = \log [(t - t_0/t_0)] = \log (t - t_0) - \log t_0$, the log t_0 term could be incorporated into the constant b. Since this value differs from column to column, the separate constant, c, is justified:

$$\log P(o/w) = (m \times \log V_r) + b + c \qquad \text{(Eq. 4)}$$

Equation 4 describes a proportionality between the retention volume of a compound and its partitioning between mobile and stationary phases. The constants m, b, and c reflect the chromatographic process, the conversion from stationary/mobile phase to octanol/water phase partitioning, and the difference between columns.

Despite problems with HPLC measurement of log P(o/w), Baker *et al.* and Henry *et al.* suggested that HPLC retention data may prove to be a better correlate of biological activity than the octanol/water partition coefficient (9, 13). In this paper, an automated log P(o/w) measurement (ALPM), based on further modification of Eqs. 3 and 4 is reported; it allows variable flow rates, temperatures, and column lengths, and compensates for column changes over time. This alteration affords a corrected retention volume proportional to log P(o/w) and enables estimation of the latter from 0 to nearly 8 units. The ALPM log P(o/w) displays high reproducibility and excellent general correlation with the shake-flask result. The subsequent paper of this series demonstrates the value of ALPM in predicting biological activities (14).

EXPERIMENTAL SECTION

Definitions—The procedure utilizes a temperature- and column-length-corrected retention volume, V_{cr}, in analogy to the definition of the retention volume (V_r) by Henry *et al.* (13). Specifically, the term $(t - t_0)$ in Eq. 3 is replaced by the value from Eq. 6, which is obtained from extrapolated measurements of percent alcohol (as the abscissa) *versus* log $(t - t_0)$ when the percent alcohol is 0, by taking the antilog of the linear relationship in Eq. 5:

$$\log (t - t_0) = [(\text{slope} \times \text{percent alcohol}) + \text{tci}] \qquad \text{(Eq. 5)}$$

$$(t - t_0) = 10^{[(\text{slope} \times \text{percent alcohol}) + \text{tci}]} \qquad \text{(Eq. 6)}$$

where: $t - t_0$ is the elution time of compound minus the elution time of the void volume marker (min); the percent alcohol is 0 (for 100% aqueous buffer); slope is the slope of log $(t - t_0)$ *versus* percent alcohol plot; tci represents the temperature-corrected intercept, i + [(CT − RT) × 0.015 per degree] where i is the intercept (0% alcohol); CT represents the column temperature; RT represents the reference temperature (for this work, RT = 25°C). Correction for different column lengths involves referencing the results to a 25-cm column. Since a shorter column would proportionally reduce V_{cr}, multiplication by the ratio (25/CL) restores V_{cr} to a constant value. Thus:

$$V_{cr} = \frac{10^{[(\text{slope} \times \text{percent alcohol}) + \text{tci}]} FR\ (25\ \text{cm})}{CL} \qquad \text{(Eq. 7)}$$

where CL is the column length (cm) and FR is the flow rate (mL/min)[1].

Log P(o/w) can be determined from Eq. 8 using the corrected retention volume, V_{cr}, from Eq. 7 and C_{cf}, a column correction factor measured in log P(o/w) units, which replaces c in Eq. 4:

$$\log P(o/w) = (m \times \log V_{cr}) + b + C_{cf} \qquad \text{(Eq. 8)}$$

Column changes are compensated by the column correction factor, C_{cf} (measurement is described below). With methanol as the alcoholic cosolvent and using a reference temperature of 25°C, two sets of constants are reported in Eqs. 9 and 10[2]. For 0.004 M trifluoroacetic acid and 0.035 M triethylamine[2]:

$$m = 1.118(\pm 0.010);\ b = -0.588(\pm 0.019);\ r^2 = 0.9988;\ SE = 0.0409 \qquad \text{(Eq. 9)}$$

For 0.015 M triethylamine[2]:

$$m = 1.169(\pm 0.032);\ b = -0.946(\pm 0.092);\ r^2 = 0.9993;\ SE = 0.0578 \qquad \text{(Eq. 10)}$$

Log V_{ccr} is defined as the C_{cf} and column-corrected equivalent to log V_{cr}:

$$\log V_{ccr} = \log V_{cr} + (C_{cf}/m) \qquad \text{(Eq. 11)}$$

Replacement of log V_{cr} in Eq. 8 by $[\log V_{ccr} - (C_{cf}/m)]$ from Eq. 11 affords a column-independent definition of chemical hydrophobicity, which can be related to log P(o/w) as in Eq. 12 with the same constants, m and b:

$$\log P(o/w) = (m \times \log V_{ccr} + b) \qquad \text{(Eq. 12)}$$

Instrumentation and Columns—All experiments were performed on a liquid chromatograph equipped with column-temperature control, automatic injector, automatic sample changer, and serial computer interface[3]. HPLC peaks were detected using either the fixed-wavelength detector at 254 nm or the variable-wavelength detector at 190–600 nm, as necessary. Retention times, computed by the HPLC hardware are independent of the volume and concentration injected from 1 to at least 30 μL for benzyl alcohol and quinoline using a 1-mg/mL solution[4]. Columns of 1, 3, 10, and 25 cm, packed with 10-μm, C_8-alkylated adsorbant, were used without modification[5].

Chemicals—HPLC-grade methanol[6] and deionized[7], distilled water were used as HPLC solvents. Nicotinic hydrazide *N*-oxide[8], pyridine *N*-oxide[8], triethylamine[8], trifluoroacetic acid[8], and tetrahydrofuran[8] were obtained commercially. The triethylamine or trifluoracetic acid, used as buffers, must be present only in the aqueous solvent reservoir to prevent rapid destruction of the HPLC ball valves. Although Mirrlees *et al.* chose pyridine *N*-oxide as the void volume marker, nicotinic hydrazide *N*-oxide elutes earlier under our conditions and is preferred (12). Chemicals were obtained from various sources[8,9] or were synthesized (15).

Procedures—Several milligrams of nicotinic hydrazide *N*-oxide and the compound to be measured were diluted to 1–2 mL with methanol. (To improve the solubility of agents with log P(o/w) values of >4.5, several microliters of saturated aqueous nicotinic hydrazide *N*-oxide can be substituted for the solid nicotinic hydrazide *N*-oxide and tetrahydrofuran can replace methanol in the injection solution.) Several microliters of the solution were injected for each analysis. The choice of organic injection solvent had no detectable effect on the log V_{cr}. Routine data analysis utilized a minicomputer and software written

[1] Although percent alcohol is zero for the determination of V_{cr} in Eq. 7, prior ALPM determinations of the log P(o/w) and the slope, and knowledge of the current value of t_0 enable Eqs. 7 and 8 to be solved for the theoretical elution time for that compound at any percent of the appropriate solvent where equilibrium conditions are maintained.

[2] Values for the constants m, b, and C_{cf} (Eq. 8) are valid only under the conditions stated here and only with Hewlett-Packard Model 1084B HPLC instruments manufactured before the June 1982 reduction in the injector system volume for narrower bore columns. Use of this procedure under the conditions stated, but with other HPLC instruments, will require the determination of new values for these constants.

[3] Model 1084B; Hewlett-Packard.

[4] Unpublished results.

[5] RP-8 Merck brand reverse-phase, silica columns; Brownlee Laboratories, Santa Clara, Calif. The RP-8 packing material used herein has been changed since this work was completed. As a result, identical columns are no longer available; but more efficient and pH-resistant C_8 types of packing could afford better results using these procedures. New regression constants must be determined, however.

[6] MCB Manufacturing Chemists Inc., Cincinnati, Ohio.

[7] Milli-Q system; Millipore Corp., Bedford, Mass.

[8] Aldrich Chemical Co., Milwaukee, Wis. Nicotinic hydrazide *N*-oxide was obtained from the Alfred Bader group of Aldrich.

[9] Chem Service Inc., West Chester, Pa.; Pfaltz and Bauer, Stamford, Conn.; K and K Laboratories, Plainview, N.Y.

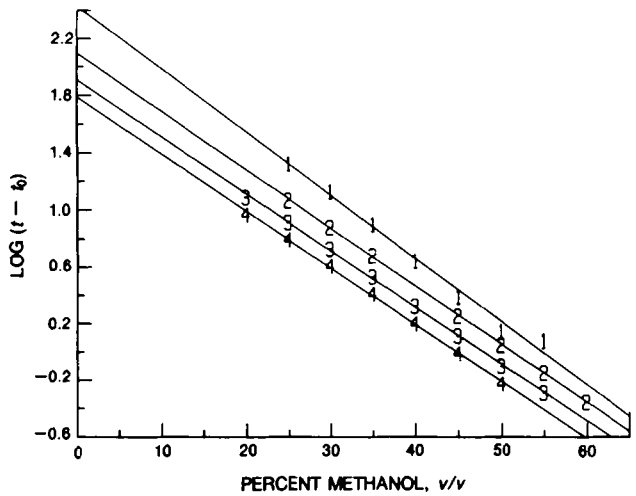

Figure 1—*Log* (t − t_0) versus *percent methanol for different flow rates. A parallel, linear relationship is apparent for all flow rates examined although statistical scatter increases using 1 mL/min for 1-(3-furyl)-4-methylpentan-1-one. Key: (1) 1 mL/min; (2) 2 mL/min; (3) 3 mL/min; (4) 4 mL/min.*

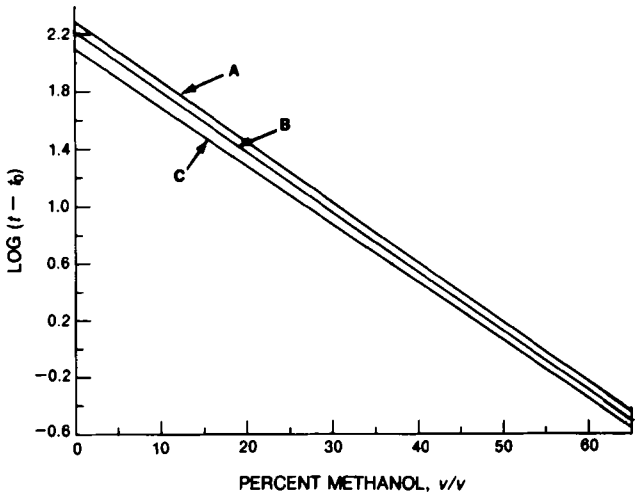

Figure 2—*Log* (t − t_0) versus *percent methanol for different temperatures. A parallel, linear relationship is apparent for temperatures from 17°C to 37°C for 1-(3-furyl)-4-methylpentan-1-one. Key: (A) 17°C; (B) 27°C; (C) 37°C.*

for this purpose[10]. When used, automatic data processing involved computer control of the chromatograph, data entry, and analysis steps[10].

Interpretation of Summary Data Tables—ALPM can produce considerable information that is helpful in evaluating the log P(o/w) results obtained for each compound. For example, some indication of HPLC equilibrium status, as discussed elsewhere, is given by these data (14)[11]. Equal values for n_0 and n indicate that all measured values were used in the linear regression process (*e.g.*, significant deviation from linearity over the measured range was not encountered). The greater the difference between n_0 and n, the more likely that curvilinearity, rather than simple statistical scatter, accounts for points being deleted from the linear regression (14). Where F_{calc} exceeds F_{set}, the data are indeed curved, but the low SE often suggests that the curve was randomly generated and need not be considered further. Increasing caution in accepting the data is urged when the SE exceeds 0.01 and/or the correlation coefficient falls below 0.999. However, the log P(o/w) may still be quite close to the literature value when high SE values and/or low correlations occur.

RESULTS

Figures 1 and 2 for 1-(3-furyl)-4-methylpentan-1-one typify log ($t - t_0$) *versus* percent methanol for different flow rates and column temperatures. The extrapolated intercept at 100% aqueous buffer was determined, corrected for measurement conditions by Eq. 7, and then converted to log P(o/w) according to Eq. 8, using the appropriate constants. The second paper in this series discusses the meaning of flow plot linearity and the extrapolation concept in greater detail (14).

Variable Flow Rates—Flow rates of 1-4 mL/min are statistically parallel for 1-(3-furyl)-4-methylpentan-1-one (Fig. 1). This is expected since flow rate and elution time are inversely related (16). All commercial HPLC instruments have some limitations. The HPLC used for these studies requires check valves to regulate the pumping process and requires at least a 10-bar column pressure to ensure correct seating of these values. The increased scatter and inaccuracy apparent at 1-mL/min flow rates for 1-(3-furyl)-4-methylpentan-1-one and naphthalene (*cf.*, Fig. 1, Table I) may arise from the low pressure generated with small columns by this low flow rate. Unrecognized instrumental limitations of this and/or other types may have contributed to past difficulties with other HPLC log P(o/w) determinations.

Temperature Variations—While temperature variations are probably not a major factor during a single run, they can affect the log V_{ccr} measurement and log P(o/w) reproducibility (Fig. 2, Table I). Data for several compounds suggest a consistent decrease in the intercept and a slight, but generally insignificant change in the slope as the temperature increases. As a result, an empirical intercept correction term, to restore the intercept to that expected at 25°C, is included in Eq. 6 and is supported by data in Table I. Column temperatures near 35°C are favored because temperature correction is empirical and valid only over a narrow range, elution times are faster, column pressures are lower, and the results are more reproducible. Once obtained, however, these data are referenced to 25°C for comparison with literature values; other reference temperatures could be used, but a new correction formula and regression constants would be needed.

Mobile Phase—The aforementioned findings have clarified the role of HPLC instrumental variables on the determination of log P(o/w), but the choice of mobile phase and column variables is also critical. The partition coefficient of most value in correlation work is that of the uncharged, neutral form, since this can exceed the ionized form by 1000-fold (2). To maintain the chemical in that form, dilute acid or base is required for acids or bases, respectively. Since halogen salts should be avoided when using stainless steel and concentrated salt solutions can ooze and block the very small-diameter tubing, miscible volatile organic acids and bases are preferred.

Although the log V_{cr} values for carboxylic acids and phenols were unaffected by concentrations of >0.004 M trifluoroacetic acid[4], base concentrations of ≫0.004 M were required. Elution of pyridine with 0.02 M Tris, trimethylamine, and triethylamine indicated that the strongest base gave the lowest log V_{ccr} measurement (*i.e.*, reduced tailing) ($r > 0.99$, Table II). As triethylamine elutes pyridine more quickly than the organic bases, it was chosen as the base modifier. Triethylammonium siloxide ion-pair formation was supported by the fact that the strongest base, NaOH, eluted pyridine slower than any of the organic bases. If a simple base interaction were involved, NaOH should have eluted pyridine before the organic bases. However, the determination with NaOH affected subsequent column performance.

The high pH of the triethylamine solution might have caused serious column degradation and only 0.015 M was used initially. However, column deterioration was less of a problem than anticipated, and the optimal concentration was determined. Triethylamine concentration *versus* log V_{cr} for pyridine (a compound very sensitive to base concentration) is shown in Fig. 3. It appears that concentrations at or somewhat above 0.06 M afford log V_{cr} values totally independent of base concentration; 0.035 M triethylamine affords greater independence of log V_{cr} from base concentration than does 0.015 M. However, the use of 0.035 M triethylamine probably offers increased column life. Although changes do occur as a result of base degradation, some columns have been used for many months by taking advantage of an external standard column factor (described below).

[10] Hewlett-Packard Model 9845B desktop computer with 187 Kbytes internal memory, internal printer, I/O-, Graphics-, Advanced Programming-, and Structured Programming ROMS, and the necessary instrument interfaces were used for data acquisition and/or analysis. A more complete description of this copyrighted ALPM software, enabling multicomputer-controlled log P(o/w) determination, computation, and comparative analysis, complete with an example, is in preparation.

[11] Condition numbers 1-4 refer to organic solvents methanol, absolute ethanol, isopropyl alcohol, or acetonitrile, respectively. n_0 and n refer to the numbers of data points actually measured and those used in the linear regression. Low, high, intercept, slope, and r relate the lowest and highest solvent percentage measurements used in the regression line, its intercept, slope, and correlation coefficient r, respectively. F_{set} indicates that table F value above which a quadratic fit is indicated (or below which the points are accepted as statistically linear). F_{calc} represents the computed F value required for a linear fit of the measured number of points, all at the 96.9% confidence level. If F_{calc} is below F_{set}, the data are not statistically curved. Choice of the unusual 96.9% confidence level for a linear relationship was based on deviations encountered with benzene (14). SE refers to the standard error of estimate of log ($t - t_0$) by linear regression on percent methanol. V_{ccr} and C_{cf} are defined as the corrected column retention volume and the column correction factor, respectively. ALPM log P and log P range refer to the octanol/water partition coefficient as measured by ALPM and the range of shake-flask log P(o/w) literature values when present. Likewise, when present, Best indicates the single literature value closest to ALPM.

Table I—Flow Rate and Temperature Comparisons with Methanol as Organic Cosolvent

Compound	Obs.	n_0	Flow Rate, mL/min	Column Length, cm	Column Serial No.	Condition	Oven Temp, °C	Intercept	Slope	Solvent Percentage Low	Solvent Percentage High	n	r	F_{set}	F_{calc}	SE	log V_{cor}	C_{cf}	ALPM log P	Best
4-Bromophenol	1	10	2	10	1349	1	27	2.055 ± 0.050	−0.038 ± 0.001	40	55	8	0.9996	8.847	4.857	0.0065	+2.78 ± 0.05	+0.00	+2.56 ± 0.06	+2.59
	2	10	2	10	1349	1	32	1.963 ± 0.049	−0.037 ± 0.001	40	55	8	0.9996	8.847	4.450	0.0064	+2.77 ± 0.05	+0.00	+2.54 ± 0.06	+2.59
	3	10	2	10	1349	1	37	1.820 ± 0.051	−0.035 ± 0.001	35	55	10	0.9993	7.246	0.459	0.0105	+2.70 ± 0.05	+0.00	+2.46 ± 0.06	+2.59
	4	10	2	10	1349	1	42	1.750 ± 0.012	−0.035 ± 0.000	40	55	8	1.000	8.847	0.244	0.0016	+2.70 ± 0.01	+0.00	+2.47 ± 0.01	+2.59
1-(3-Furyl)-4-methylpentan-1-one	5	7	1	3	0000	2	37	2.432 ± 0.236	−0.044 ± 0.006	25	55	7	0.9942	10.646	1.296	0.0570	+3.53 ± 0.24	+0.00	+3.17 ± 0.28	—
	6	8	2	3	0000	2	17	2.289 ± 0.043	−0.042 ± 0.001	25	60	8	0.9997	8.847	1.172	0.0126	+3.39 ± 0.04	+0.00	+3.00 ± 0.05	—
	7	7	2	3	0000	2	27	2.207 ± 0.037	−0.042 ± 0.001	25	60	7	0.9999	10.646	0.272	0.0101	+3.46 ± 0.04	+0.00	+3.08 ± 0.04	—
	8	7	2	3	0000	2	37	2.097 ± 0.032	−0.041 ± 0.001	25	60	7	0.9999	10.646	0.143	0.0087	+3.50 ± 0.03	+0.00	+3.13 ± 0.04	—
	9	8	3	3	0000	2	37	1.911 ± 0.046	−0.040 ± 0.001	20	55	8	0.9996	8.847	8.318	0.0149	+3.49 ± 0.05	+0.00	+3.12 ± 0.05	—
	10	9	3	3	0000	2	37	1.920 ± 0.035	−0.040 ± 0.001	20	60	9	0.9997	7.862	7.139	0.0134	+3.50 ± 0.04	+0.00	+3.13 ± 0.04	—
	11	7	4	3	0000	2	37	1.896 ± 0.024	−0.043 ± 0.001	35	55	5	1.0000	30.766	7.868	0.0025	+3.60 ± 0.02	+0.00	+3.25 ± 0.03	—
Naphthalene	12	12	1	3	0001	3	35	2.334 ± 0.020	−0.037 ± 0.000	30	55	12	0.9999	6.522	5.607	0.0059	+3.45 ± 0.02	+0.05	+3.30 ± 0.02	+3.45
	13	12	2	3	0001	3	35	2.113 ± 0.017	−0.038 ± 0.000	30	55	12	0.9999	6.522	2.821	0.0051	+3.53 ± 0.02	+0.05	+3.38 ± 0.02	+3.45
	14	10	3	1	0101	3	35	1.558 ± 0.020	−0.038 ± 0.000	30	50	10	0.9999	7.246	3.275	0.0047	+3.58 ± 0.02	+0.00	+3.44 ± 0.02	+3.45
	15	14	3	1	0102	3	35	1.589 ± 0.017	−0.039 ± 0.000	30	50	10	0.9999	7.246	4.082	0.0040	+3.58 ± 0.02	−0.04	+3.44 ± 0.02	+3.45
	16	12	3	3	0001	3	35	1.976 ± 0.019	−0.039 ± 0.000	30	55	12	0.9999	6.522	0.713	0.0056	+3.57 ± 0.02	+0.05	+3.43 ± 0.02	+3.45
	17	9	4	3	0001	3	15	2.236 ± 0.032	−0.042 ± 0.001	30	55	8	0.9998	8.847	5.745	0.0066	+3.61 ± 0.03	+0.00	+3.47 ± 0.04	+3.45
	18	12	4	3	0001	3	25	2.078 ± 0.023	−0.041 ± 0.001	30	55	12	0.9998	6.522	0.738	0.0068	+3.60 ± 0.02	+0.00	+3.46 ± 0.03	+3.45
	19	12	4	3	0001	3	35	1.896 ± 0.019	−0.039 ± 0.000	30	55	12	0.9999	6.522	2.685	0.0056	+3.57 ± 0.02	+0.00	+3.43 ± 0.02	+3.45
	20	12	4	3	0001	3	45	1.776 ± 0.025	−0.038 ± 0.001	30	55	12	0.9998	6.522	4.740	0.0075	+3.60 ± 0.03	+0.00	+3.46 ± 0.03	+3.45

[a] See footnote 11 (in text) for definitions.

Table II—Log V_{ccr} of Pyridine *versus* 0.02 M Base

Base	Log V_{ccr}	pK_b
Organic		
Tris	1.69	5.92
Trimethylamine	1.38	4.13
Triethylamine	1.22	2.99
Inorganic		
Sodium hydroxide	1.83	~0

When 0.035 M triethylamine is used for bases and neutral compounds and 0.004 M trifluoroacetic acid is used for acidic compounds, the acid and base least-squares linear correlation equations become statistically indistinguishable in the range of 0.3–6 log P(o/w) units, and probably higher. This effect is dramatically exemplified by the relationship between log V_{ccr} and log P(o/w) (Fig. 4). The upper line in Fig. 4 represents the acidic and numerous basic or neutral compounds; the lower line depicts basic or neutral compounds measured in 0.015 M triethylamine. As triethylamine levels increase, the lower line in Fig. 4 merges with the upper line. Triethylamine at 0.06 M may prove statistically valuable for the most accurate determinations below 0.3 log P(o/w) units (Fig. 3). This is also evidenced by the very slight deviation of the overall column-corrected log P(o/w) *versus* literature correlation from a zero intercept as indicated in Fig. 5. Equations 8–12 describe the regression equations relating log P(o/w) and/or log V_{cr} for the different acid and base concentrations.

Several nonaqueous mobile phases were also studied. Figure 6 is a plot of percent cosolvent *versus* log $(t - t_0)$ using four different organic solvents and 0.015 M triethylamine to elute 1-(3-furyl)-4-methylpentan-1-one. Note that different slopes and extrapolated intercepts are obtained for these lines; this suggests that a solvent-strength-related intercept conversion factor could allow measurement of higher log P(o/w) values in other alcohols. Although methanol was chosen as the organic mobile phase for economic reasons, isopropyl alcohol may facilitate determination of even higher values, despite increased slopes and the resultant increase in the log P(o/w) error. Nonalcoholic solvents, such as acetonitrile, may distort the linear response of log $(t - t_0)$ *versus* percent solvent and complicate the conversion process (14).

Column Variables—The HPLC column is also critical to ALPM. The following column lengths afford an optimum elution time for the various log P(o/w) ranges: 0–~0.8, 50 cm; ~0.3–~1.5, 25 cm; ~1–~3, 10 cm; ~2.2–~5, 3 cm; and >~5, 1 cm. However, compounds run on different columns or the next longer or shorter column length give statistically the same results (Table I: obs. 15 and 16; Table III: obs. 22 and 23, 25 and 26, 39 and 40, and 62 and 63). These data indicate that only two or three column lengths are required. Despite the fact that ALPM has enabled measurements as high as 7.8 for hexabromobiphenyl (14), existing shake-flask methods cannot ensure that values of >6 log P(o/w) units are not low; therefore, some uncertainty does exist in fitting very high ALPM values (17). Although ALPM values for anthracene, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), and diethylstilbesterol are only slightly higher than the shake-flask result, ALPM values for phenylphosphonothioic acid *O*-ethyl *O*-*p*-nitrophenyl ester (EPN), [2.2]paracyclophane, and progesterone are considerably higher (*cf.* Table V of Ref. 14).

Column-length differences are readily corrected by Eq. 7, except when using a 1-cm column or when the column has deteriorated. A packing error of 0.1 mm becomes important with a 1-cm column and requires the use of the column

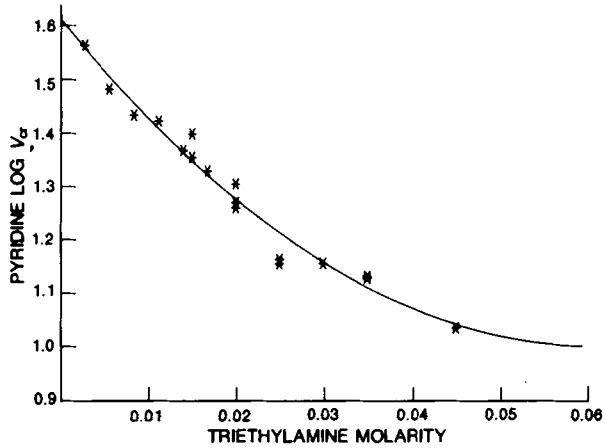

Figure 3—*Log* V_{cr} versus *triethylamine concentration. Dependence of pyridine log* V_{cr} *on triethylamine concentration becomes insignificant at* $\geq 0.06 M$.

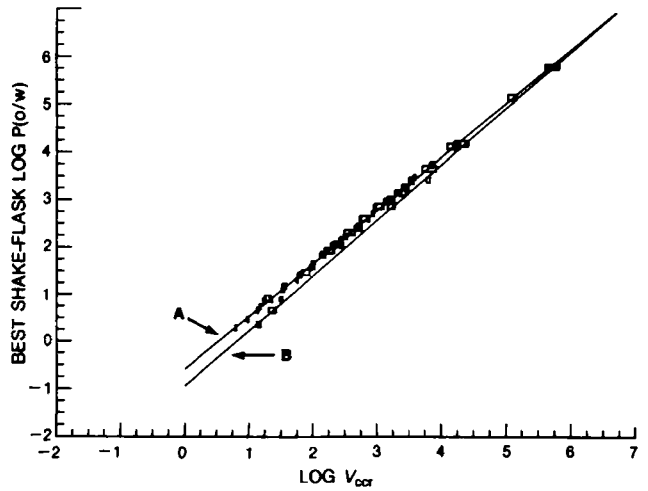

Figure 4—*Correlation between shake-flask log P(o/w) and ALPM log V_{ccr}. Although the correlation of acids is independent of concentration, the correlation of bases and neutral agents is dependent on triethylamine concentration. The boxes represent the 95% confidence limits (±2 SD) in ALPM log V_{ccr} (mean SE = ±0.049) and the mean 67% confidence limit (±SD) in log P(o/w). Key: (A) acidic compounds in 0.004 M trifluoroacetic acid; basic compounds in 0.035 M triethylamine (n = 58, r = 0.9994); (B) basic and neutral compounds in 0.015 M triethylamine (n = 8, r = 0.9996).*

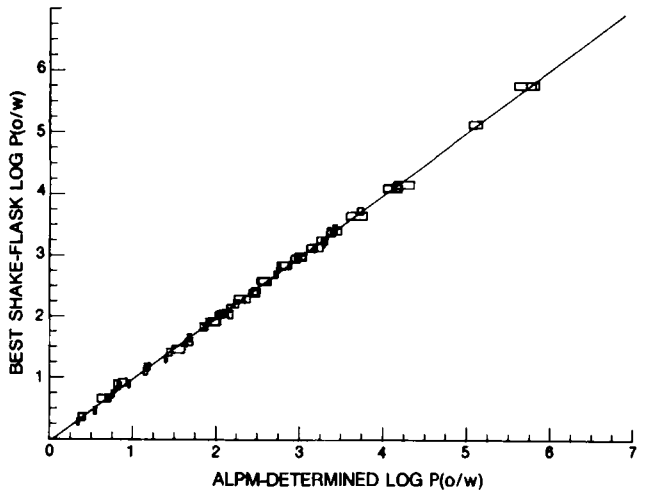

Figure 5—*Correlation between shake-flask and ALPM log P(o/w). Error boxes indicate the variable 95% confidence limits (±SD) (mean = 0.044) in shake-flask log P(o/w) as determined from the compounds shown in Table IV (n = 66, r = 0.9994). The SE of the estimate is 0.045.*

correction factor, C_{cf}. C_{cf} is determined by measuring the log P(o/w) of several agents with otherwise reproducible values. With these agents, any consistent error in log P(o/w) measured at essentially the same time is assigned to C_{cf}. Records normally include the time of C_{cf} assignment, which is checked and updated frequently. Column correction appears to be a valid technique as long as double peaks do not complicate the computation of retention times.

In early work, column deterioration frequently occurred when changing acid-base conditions. Although changing from organic acid to organic base posed few problems, changing from organic base to organic acid consistently destroyed columns. Hypothetically, changing from ion-paired triethylamine to trifluoroacetic acid could result in chemical reactions and/or heat liberation disrupting the column packing. Prior thorough washing with distilled, deionized water failed to prevent column deterioration. Therefore, columns of each length should be dedicated to each acid or base condition to maximize column life.

Changes in column C_{cf} values occur most under basic conditions. The greatest alterations occur when switching to a column that has not been used for an extended period, suggesting that base degradation may be a slow, continuous process. Nevertheless, such changes can be corrected using the C_{cf}; but, in contrast to the 1-cm columns, the C_{cf} for longer columns generally should not be negative, because overpacking is not a problem[12].

DISCUSSION

The value of the partition coefficient to pharmacology and toxicology arises from the dependence of Fick's permeability constant on the partition coefficient (18). As a result, the partition coefficient [*i.e.*, log P(o/w)] governs the absorption, distribution, metabolism, and excretion of chemicals (18-20). Many HPLC procedures that measure log P(o/w) have distinct advantages over shake-flask methods. HPLC procedures provide a continuous scale for measurement of both low and high partition coefficients and offer greater assurance that spectroscopic detection monitors component X, rather than highly absorbent trace impurities (9). In addition, HPLC measurement of elution times can be relatively fast and the conditions carefully controlled (12). HPLC procedures utilize lower concentrations of the agent of interest and may better avoid disequilibria (*e.g.*, multiple components), but HPLC elution time measurements, made as a function of solvent composition, may identify such disequilibria should they occur. The same assurance for shake-flask studies requires, at minimum, the repetition of log P(o/w) measurements at different concentrations and extrapolation to infinite dilution. HPLC methods can also enable partition coefficient determination when compounds are sufficiently unstable as to prevent their determination by conventional shake-flask techniques (21).

Despite these advantages, most available HPLC methods rarely cover much more than 3-3.5 log P(o/w) units (12, 13, 22, 23). Previous HPLC log P(o/w) methods have made use of C_{18} reverse-phase silica columns (9, 22) and/or packing materials coated with octanol to make the columns more "octanol-like" (12, 13, 23, 24). Some still gave poor correlations between elution data and literature or measured log P(o/w) values (13, 25). A relatively recent HPLC method by Veith *et al.* affords a large range of log P(o/w) (26). It uses a curvilinear fit between log P(o/w) and an elution parameter obtained from a programmed nonlinear increase in HPLC flow rate, but even this method gives substantial unexplained deviations from a smooth curve.

ALPM differs in several respects from the other HPLC procedures. ALPM uses C_8 reverse-phase columns, which contain greater hydrocarbon surface areas than either the equivalent C_2 or C_{18} packings (27). Besides using a different alkyl group length, ALPM gives greater assurance than shake-flask or other HPLC methods that the partitioning process achieves the equilibrium so essential to accurate determinations (14). Judicious choices of flow rate, column temperatures, and column lengths allow compounds of widely differing lipophilicities to elute within 10 min. Previously, only Henry *et al.* and Veith *et al.* utilized variable flow rates to expedite the elution process (13, 26). Rapid elution of peaks by ALPM retains excellent peak shapes, which facilitate accurate elution times.

The logarithm of the corrected retention volume (V_{cr}) first obtained by ALPM is converted by linear regression to log P(o/w) values based on the shake-flask procedure. The discovery that two distinct lines (*e.g.*, Fig. 4)

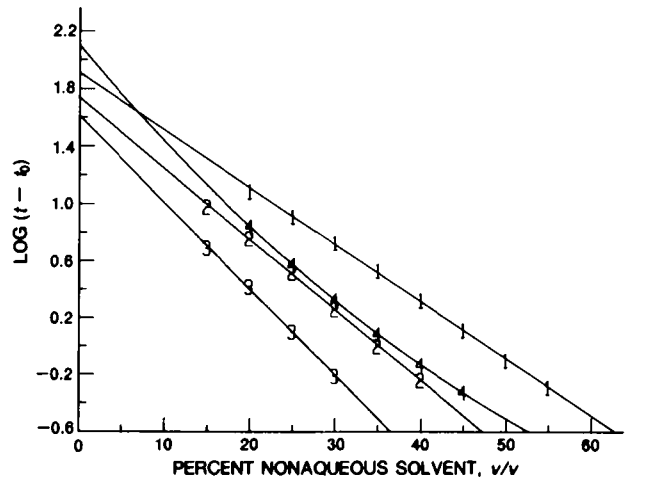

Figure 6—*Log $(t - t_0)$ versus cosolvent. Alcohols afford a linear relationship, although the slopes differ considerably for 1-(3-furyl)-4-methylpentan-1-one. Nonalcoholic solvents, however, do not always give a linear relationship. Key: (1) methanol; (2) absolute ethanol; (3) isopropyl alcohol; (4) acetonitrile.*

[12] Using one 10-cm column, however, it became apparent from several prior determinations using several different compounds, that the column C_{cf} had changed substantially from a positive (*i.e.*, needing a correction) to a negative (*i.e.*, being overcorrected) value. An explanation of this C_{cf} reversal is unclear, but its occurrence is noteworthy.

Table III—HPLC Log P(o/w) Standards using Methanol as Cosolvent [a]

Compound	Obs	n_0	Flow Rate, mL/min	Column Length, cm	Column Serial Number	Condition	Oven Temp., °C	n	r	log V_{ccr}	C_{cf}	ALPM log P	log P Range	Best
Acetanilide	1	19	2	10	1516	3	35	14	0.9998	+1.54 ± 0.01	+0.00	+1.18 ± 0.01	+1.16 to +1.36	+1.17
	2	9	2	10	7172	3	32	9	0.9993	+1.53 ± 0.02	+0.09	+1.17 ± 0.03	+1.16 to +1.36	+1.17
	3	8	2	10	9412	3	34	8	0.9998	+1.55 ± 0.02	+0.00	+1.19 ± 0.02	+1.16 to +1.36	+1.17
Acetophenone	4	10	2	10	1361	3	37	10	0.9998	+1.99 ± 0.02	+0.10	+1.68 ± 0.02	+1.58 to +1.73	+1.65
Acridine	5	14	2	1	0102	3	35	12	0.9999	+3.50 ± 0.02	−0.04	+3.35 ± 0.02	+3.40	+3.40
	6	7	4	3	0000	2	37	7	0.9999	+3.79 ± 0.03	+0.00	+3.47 ± 0.03	+3.40	+3.40
4-Aminopyridine	7	11	2	25	8390	3	35	11	0.9971	+0.79 ± 0.01	+0.00	+0.34 ± 0.01	+0.28	+0.28
Aniline	8	6	2	25	0000	2	37	6	0.9999	+1.49 ± 0.02	+0.00	+0.79 ± 0.02	+0.85 to +0.98	+0.89
	9	7	4	25	8390	3	35	7	0.9999	+1.35 ± 0.00	+0.00	+0.96 ± 0.01	+0.85 to +0.98	+0.89
Anisole	10	7	3	10	1516	3	35	6	0.9996	+2.34 ± 0.04	+0.00	+2.06 ± 0.04	+2.04 to +2.11	+2.04
Benzoic acid	11	6	3	10	1516	1	37	6	0.9999	+2.44 ± 0.03	+0.00	+2.18 ± 0.03	+1.87 to +2.03	+2.03
Benzonitrile	12	12	2	10	1361	3	37	12	0.9999	+1.96 ± 0.01	+0.10	+1.65 ± 0.01	+1.56	+1.56
Benzyl alcohol	13	7	2	10	9412	3	37	7	0.9997	+1.52 ± 0.02	+0.10	+1.16 ± 0.02	+1.10	+1.10
Biphenyl	14	6	4	3	0001	3	35	6	0.9997	+4.18 ± 0.10	+0.00	+4.11 ± 0.11	+3.16 to +4.17	+4.09
4-Bromoacetanilide	15	10	3	10	1346	3	37	9	0.9997	+2.62 ± 0.03	+0.22	+2.37 ± 0.04	+2.29	+2.29
	16	10	3	10	1361	3	37	8	0.9993	+2.54 ± 0.06	+0.00	+2.29 ± 0.06	+2.29	+2.29
4-Bromoaniline	17	12	3	10	1346	3	37	10	0.9998	+2.36 ± 0.02	+0.00	+2.09 ± 0.02	+2.05 to +2.26	+2.05
	18	12	3	10	9412	3	37	10	0.9994	+2.37 ± 0.04	+0.10	+2.10 ± 0.04	+2.05 to +2.26	+2.05
Bromobenzene	19	8	3	3	0001	3	35	6	0.9997	+3.19 + 0.05	+0.00	+3.01 ± 0.06	+2.99	+2.99
4-Bromophenol	20	12	3	10	1349	1	37	8	0.9995	+2.79 ± 0.06	+0.00	+2.56 ± 0.07	+2.59 to +2.74	+2.59
4-Chloroaniline	21	10	2	10	1346	3	37	10	0.9998	+2.17 + 0.02	+0.22	+1.88 ± 0.02	+1.83	+1.83
Chlorobenzene	22	12	4	3	0001	3	35	10	0.9989	+3.04 ± 0.06	+0.00	+2.84 ± 0.06	+2.18 to +2.84	+2.84
	23	5	4	10	1516	3	35	5	0.9996	+3.01 ± 0.07	+0.00	+2.81 ± 0.08	+2.18 to +2.84	+2.84
4-Chlorophenol	24	8	2	10	9412	1	37	7	0.9995	+2.70 ± 0.06	+0.10	+2.46 ± 0.06	+2.35 to +2.53	+2.39
3-Cyanopyridine	25	8	2	25	0000	2	37	7	0.9995	+1.14 ± 0.03	+0.00	+0.38 ± 0.03	+0.36	+0.36
	26	6	2	50	0000	2	37	6	0.9995	+1.15 ± 0.03	+0.00	+0.39 ± 0.04	+0.36	+0.36
4-Cyanopyridine	27	7	2	25	8390	3	35	7	0.9996	+0.97 ± 0.01	+0.00	+0.55 ± 0.02	+0.46	+0.46
1,2-Dibromobenzene	28	5	4	3	0001	3	35	4	0.9999	+3.80 ± 0.11	+0.00	+3.68 ± 0.12	+3.64	+3.64
1,4-Dichlorobenzene	29	9	4	3	0001	3	35	9	0.9996	+3.52 ± 0.04	+0.00	+3.37 ± 0.05	+3.37 to +3.38	+3.37
1,2-Dihydroxybenzene	30	8	2	25	9154	1	35	8	0.9987	+1.28 ± 0.04	+0.00	+0.88 ± 0.04	+0.84 to +1.01	+0.92
2,4-Dimethylphenol	31	10	3	10	1349	1	37	10	0.9997	+2.70 ± 0.03	+0.00	+2.47 ± 0.04	+2.30 to +2.42	+2.42
Diphenylamine	32	12	4	3	0001	3	35	11	0.9998	+3.84 ± 0.03	+0.05	+3.72 ± 0.03	+3.22 to +3.72	+3.72
Diphenylnitrosamine	33	10	4	10	1361	3	37	6	0.9996	+3.35 ± 0.08	+0.10	+3.18 ± 0.09	+3.13	+3.13
4-Ethylphenol	34	10	2	10	1349	1	37	7	0.9995	+2.81 ± 0.07	+0.00	+2.59 ± 0.07	+2.26 to 2.58	+2.58
3-Fluoroaniline	35	12	2	10	1361	3	37	12	0.9998	+1.74 ± 0.01	+0.10	+1.40 ± 0.01	+1.30	+1.30
Hexachlorobenzene	36	5	4	3	0000	2	37	5	0.9999	+5.70 ± 0.11	+0.00	+5.70 ± 0.12	+5.75	+5.75
	37	4	4	3	0001	3	35	4	1.0000	+5.70 ± 0.07	+0.00	+5.79 ± 0.07	+5.75	+5.75
Indole	38	10	3	10	1346	3	37	10	0.9997	+2.43 ± 0.02	+0.22	+2.16 ± 0.03	+2.00 to 2.25	+2.14
Iodobenzene	39	13	3	1	0101	3	35	9	0.9999	+3.44 ± 0.02	−0.10	+3.29 ± 0.03	+3.25	+3.25
	40	6	3	3	0001	3	35	6	0.9996	+3.43 ± 0.06	+0.00	+3.27 ± 0.06	+3.25	+3.25
4-Methoxyaniline	41	8	2	25	8390	3	37	8	0.9995	+1.23 ± 0.02	+0.00	+0.83 ± 0.02	+0.78 to 0.95	+0.86
3-Methoxyphenol	42	10	2	10	1349	1	37	10	0.9994	+1.99 ± 0.03	+0.00	+1.68 ± 0.04	+1.58	+1.58
4-Methylaniline	43	10	2	10	1361	3	37	8	0.9995	+1.78 ± 0.03	+0.10	+1.44 ± 0.03	+1.39 to +1.41	+1.41
4-Methylphenol	44	10	2	10	1349	1	37	8	0.9991	+2.27 ± 0.06	+0.00	+1.98 ± 0.07	+1.92 to +1.99	+1.92
Naphthalene	45	12	3	3	0001	3	35	12	0.9999	+3.57 ± 0.02	+0.05	+3.43 ± 0.02	+3.01 to +3.59	+3.45
1-Naphthol	46	4	4	10	1516	1	37	4	1.0000	+3.22 ± 0.04	+0.00	+3.04 ± 0.04	+2.31 to +2.98	+2.98
	47	8	4	10	9412	1	37	6	0.9999	+3.16 ± 0.03	+0.10	+2.98 ± 0.03	+2.31 to +2.98	+2.98
Nitrobenzene	48	10	2	10	1346	3	37	10	0.9998	+2.13 ± 0.02	+0.22	+1.83 ± 0.02	+1.79 to +1.88	+1.83
4-Nitrophenol	49	12	2	10	1349	1	37	8	0.9989	+2.24 ± 0.07	+0.00	+1.96 ± 0.08	+1.38 to +2.08	+1.91
Pentachlorophenol	50	8	4	3	0001	1	40	8	0.9997	+5.08 ± 0.07	+0.00	+5.11 ± 0.07	+5.01 to +5.12	+5.12
Phenazine	51	5	2	3	0000	2	30	5	0.9998	+3.22 ± 0.05	+0.00	+2.80 ± 0.06	+2.84	+2.84
Phenol	52	5	2	10	1516	1	37	5	0.9995	+1.88 ± 0.07	+0.00	+1.55 ± 0.07	+1.46 to +1.51	+1.46
Phenothiazine	53	10	4	3	0001	3	35	7	0.9993	+4.30 ± 0.11	+0.05	+4.24 ± 0.12	+3.78 to +4.15	+4.15
Phenylthiourea	54	12	3	25	8390	3	35	10	0.9997	+1.16 ± 0.01	+0.00	+0.76 ± 0.02	+0.73	+0.73
Pyridine	55	7	3	25	0000	2	37	5	0.9993	+1.36 ± 0.05	+0.00	+0.63 ± 0.06	+0.64 to +0.66	+0.66
	56	12	3	25	8390	3	37	12	0.9991	+1.13 ± 0.01	+0.00	+0.72 ± 0.02	+0.64 to +0.66	+0.66
	57	4	4	25	0000	2	37	4	1.0000	+1.40 ± 0.02	+0.00	+0.68 ± 0.02	+0.64 to +0.66	+0.66
	58	6	4	25	8390	3	35	6	0.9998	+1.13 ± 0.01	+0.00	+0.72 ± 0.02	+0.64 to +0.66	+0.66
Quinoline	59	12	3	10	1346	3	37	11	0.9998	+2.29 ± 0.02	+0.00	+2.01 ± 0.02	+2.02 to +2.06	+2.02
Salicylic acid	60	5	3	10	1516	1	37	5	0.9999	+2.51 ± 0.03	+0.00	+2.25 ± 0.03	+2.21 to +2.26	+2.21
Toluene	61	12	4	10	1346	3	37	12	0.9998	+2.93 ± 0.02	+0.22	+2.72 + 0.02	+2.11 to +2.80	+2.69
2,4,6-Tribromophenol	62	10	3	1	0201	1	37	10	0.9996	+4.13 ± 0.05	+0.00	+4.05 ± 0.05	+3.96 to +4.23	+4.10
	63	5	4	3	0000	1	37	4	1.0000	+4.23 ± 0.05	+0.00	+4.16 ± 0.05	+3.96 to +4.23	+4.10
3-Trifluoromethylphenol	64	10	3	10	1349	1	37	8	0.9997	+3.14 ± 0.05	+0.00	+2.95 ± 0.05	+2.95 to +3.17	+2.95
2-Xylene	65	9	3	3	0002	3	37	9	0.9999	+3.35 ± 0.02	+0.00	+3.18 ± 0.02	+2.77 to +3.12	+3.12
3-Xylene	66	10	3	3	0002	3	37	10	1.0000	+3.44 ± 0.01	+0.00	+3.28 ± 0.01	+3.20	+3.20

[a] See footnote 11 (in text) for definitions.

correlate acidic and basic chemicals has been previously reported (24, 25). However, conditions, in which reverse-phase columns offer a single linear correlation between log P(o/w) and HPLC data have not yet been elucidated. Hexabromobiphenyl afforded an ALPM log P(o/w) value of 7.8, the highest value yet measured (14). For neutral compounds, log P(o/w) measurement could be made under either acid- or base-elution conditions. Comparison of acid and base conditions for several neutral chemicals suggests occasional differences can occur (14). Base conditions seem favored for the measurement of the log P(o/w) of neutral compounds due to the substantial linearity already observed under these conditions, but this is currently under investigation.

ALPM reproducibility averages 1.06 ± 0.46% (*SD*) of the average HPLC measured value over 54 runs (Table IV). Errors in these measurements appear related to the slope of the individual log $(t - t_0)$ *versus* percent methanol lines. Although they increase as the log P(o/w) increases, they change little as a percentage of the log P(o/w) value. Reproducibility of the literature shake-flask values for virtually the same compounds averages 6.13 ± 2.76% (*SD*)

Table IV—Reproducibility of Log P(o/w) Measurements [a]

Compound	Runs	ALPM Average ± SD	Measurements	Shake-Flask Average ± SD
Acetanilide	15	1.18 ± 0.01	3	1.23 ± 0.11
Acetophenone	2	1.67 ± 0.01	2	1.66 ± 0.08
Biphenyl	2	4.12 ± 0.01	2	3.88 ± 0.41
4-Bromoaniline	8	2.07 ± 0.02	8	2.15 ± 0.15
4-Bromophenol	7	2.52 ± 0.04	7	2.60 ± 0.13
3-Chlorophenol	3	2.37 ± 0.02	3	2.49 ± 0.02
4-Chlorophenol	3	2.39 ± 0.06	3	2.42 ± 0.07
Naphthalene	9	3.42 ± 0.03	9	3.35 ± 0.19
1-Naphthol	2	3.01 ± 0.04	2	2.71 ± 0.35
Pentachlorobenzene	3	5.18 ± 0.06	3	4.94[b] —
Pentachlorophenol	1	5.11[b] —	1	5.33 ± 0.46
ALPM log P(o/w)		average error = 1.06% (±0.46%) for 54 values		
Shake-flask log P(o/w)		average error = 6.13% (±2.76%) for 40 values		

[a] The error in ALPM increases as the slope of log $(t - t_0)$ *versus* percent methanol becomes more negative. The average error is expressed as a percent of the log P(o/w) value for both the ALPM and shake-flask results. The shake-flask data are from Refs. 2, 3, and 26. [b] Not included in error determination.

of the measured value. Table III summarizes typical information obtained by ALPM for the standards used in Figs. 4 and 5.

In addition to high accuracy and excellent reproducibility, measurement of log P(o/w) by this procedure is less expensive and safer than by other methods. The shorter columns required for the majority of agents are 10–50% as expensive as 25-cm columns and generate much lower back-pressures. Neglecting the instrumentation cost, samples can be processed for ~$10.00 (which compares well with the cost of calculated values), yet the results provide as good or better a correlation with biological data (14). Even differences between isomers can be determined. All HPLC methods avoid the cumbersome use of often leaky separatory funnels and traditional UV-visible spectrophotometric analysis, with its manipulation of standards and analysis solutions; but ALPM also avoids exposure to toxicants. Samples can be prepared in a glove box and automatically injected under computer control, with wastes delivered directly into approved waste containers.

For maximal statistical validity and accuracy, ALPM requires numerous elution-time measurements. The error in ALPM log P(o/w) generally decreases as the number of measurements increase, so the needed number of determinations must be based on user requirements. An entire computer-operated run can generally be completed within 1 h for six measurements from ~55 to 30% methanol (5% increments) in descending order or within ~1.5 h for duplicate data measured first in descending, and then ascending, order. Facts pertinent to the choice of the methanol percentage range are presented in the following paper (14). Although somewhat more time is required for ALPM than for single-measurement HPLC procedures, labor expenses can be virtually eliminated by this computer-controlled analysis; it can automatically process up to 24 single or 12 duplicate runs while operating day and night.

The chemical need not be pure to use computer-data acquisition, but other components must provide smaller peak areas than either the void volume marker or the compound of interest. This purity limitation involves only the computer assignment of t and t_0. If the compound does not meet that requirement, log P(o/w) may still be measured, provided that the component peaks and times can be manually identified, but elution times must be entered into the computer *via* the keyboard. Less-obvious advantages of this desk-top-computer control include: routines for the unattended monitoring and response to HPLC-operational problems; recovery from power outages; versatile alphabetical data file listing (*e.g.* Table I); and a data format usable with commercial statistical and graphics packages.

Greater detection selectivity can be achieved for certain chemical by using variable-wavelength or fluorescence detectors. Refractive index (RI) or conductivity detectors should work, but as they measure changes instead of actual values, a given peak can be positive or negative. As a result, automated computer-data acquisition can become troublesome. Moreover, because of their enhanced sensitivity, these detectors also raise more questions about the identity of the eluting agent than do variable-wavelength detectors.

ALPM represents a major improvement in log P(o/w) measurement technology, especially for those requiring values for many compounds. It is not, however, without some problems. Other than the obvious requirements that the substance be stable in the injection solvent under the measurement conditions for the period of detection, only log P(o/w) values above zero may be measured currently. Second, log P(o/w) standards must be run externally before and/or after the compound of interest because no method is available to elucidate the peak of interest or a simultaneously measured standard. Third, ALPM fails for zwitterionic compounds which elute immediately in either acid or base and could afford incorrect values for compounds with complex, multiple pK values. Fourth, HPLC measurement of log P(o/w) values >~8 units becomes increasingly difficult. These compounds often have poor solubility even in tetrahydrofuran, and the high negative slopes encountered with these agents leads to peak broadening when higher percentages of aqueous buffer are used. This broadening diminishes the accuracy of HPLC-computed elution times and can even affect peak detection. As a result, high log P(o/w) compounds limit the range of percentages over which good data can be obtained and raise the absolute log P(o/w) error accordingly. The necessary use of the shortest columns and high flow rates for compounds with high log P(o/w) values also hastens elution of the void volume marker peak. This can cause computer-processing difficulties if that critical peak is missed or incorrectly assigned. Such problems are readily evident and, since the HPLC output is available, the data can be manually corrected often with no loss of information.

During the course of this study, several alternative uses of ALPM became apparent. Because elution times of organic acids are essentially independent of acid concentration, and 0.004 M trifluoroacetic acid has minimal effect on column life, this procedure should provide an excellent, reproducible, and automated method for quality control of RP-8, and likely all reverse-phase HPLC columns, once the necessary constants m, b, and C_{cf} are determined.

ALPM may also become a valuable addition to qualitative-quantitative analysis and structure assignment. For example, reaction of phenylmagnesium bromide with 3-cyanofuran gave two products after acid hydrolysis, which were not separable by simple vacuum distillation. We have found that treatment of other simple Grignard reagents with 3-cyanofuran gives no such problem. ALPM log P(o/w) analysis confirmed the suspected identity of the unwanted component as biphenyl, a well-known phenylmagnesium bromide degradation product.

Yet another use of ALPM technology may be automated method development and analysis. Specifically, overlap plotting of individual log $(t - t_0)$ *versus* percent solvent data for each compound can indicate the single percentage of solvent that allows easy separation of the compounds. Furthermore, once the log P(o/w) of a compound, the elution time (t_0) of the void volume marker, and the column correction factor (C_{cf}) are known, the elution time for a given agent may be computed for various aqueous-buffer methanol compositions.

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ACKNOWLEDGMENTS

This work was described at the February 1982 meeting of the Society of Toxicology in Boston, Mass. and the March 1984 Pittsburgh Conference in Atlantic City, N.J.

The authors appreciate financial support by the University of Illinois Research Board, the Biomedical Support Group (BRSG-207-RR07038), and the U.S. Government (Hatch 20-323 and NIEHS 02193). Statistical assistance by D. Gianola and the late H. W. Norton is also appreciated.

Accurate, Wide-Range, Automated, High-Performance Liquid Chromatographic Method for the Estimation of Octanol/Water Partition Coefficients II: Equilibrium in Partition Coefficient Measurements, Additivity of Substituent Constants, and Correlation of Biological Data

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Received March 7, 1983, from the *Department of Animal Science, University of Illinois, 126 Animal Sciences Laboratory, 1207 West Gregory Drive, Urbana, IL 61801.* Accepted for publication November 29, 1983.

Abstract □ Occasionally, results from the highly reproducible automated log P(o/w) measurement (ALPM) differ from those determined by shake-flask methods. Several specific examples affording different values are presented. One source of these differences may be curvilinearity in plots of log $(t - t_0)$ *versus* percent methanol, which complicate accurate intercept determinations and, thus, estimates of log P(o/w). Other sources of these differences are presented and discussed, although their cause remains unclear. Equilibrium ALPM log P(o/w) measurements of various phenyl-, methyl-, fluoro-, chloro-, and bromobenzenes, suggest substituent constants are not strictly additive. Moreover, the higher values indicate that calculated values may not be accurate for those compounds having multiple substituents or high log P(o/w) values. ALPM gives better predictability of the *in vivo* concentration process of 8 or 12 toxicants in fish than the shake-flask method, another HPLC method, or even calculated log P(o/w) values. However, it equally correlates the binding to bovine serum albumin by 34 chemicals as predicted by a combination of shake-flask and calculated log P(o/w) values reported elsewhere.

Keyphrases □ HPLC—octanol/water partition coefficient, equilibrium □ Partition coefficients—octanol/water, HPLC, equilibrium, biological correlation

An automated log P(o/w) measurement (ALPM) has been developed that utilizes high-performance liquid chromatography (HPLC) to accurately and reproducibly estimate the logarithm of the octanol/water partition coefficient at costs comparable with computation (1). ALPM differs from earlier HPLC procedures in that variable column lengths, flow rates, and temperatures have enabled determinations over the 0–8 log P(o/w) range (1). Most previous HPLC log P(o/w) procedures have involved measurement of the capacity factor, k', using a single determination at a fixed composition of water, buffer, or a low percentage of alcohol in water or buffer (2, 3):

$$k' = (t - t_0)/t_0 \qquad \text{(Eq. 1)}$$

where t and t_0 refer to the elution time of the compound of interest and void volume marker, respectively. From that data log P(o/w) has been calculated by:

$$\log P(o/w) = (m \times \log k') + b \qquad \text{(Eq. 2)}$$

Determination of log P(o/w) by shake-flask techniques requires that equilibrium be established between the aqueous and octanol phases for the single component being measured (1). Equilibrium is essential even when partitioning occurs between HPLC phases. To date, no HPLC log P(o/w) method has provided solid evidence that equilibrium is attained during the elution process. Theoretically, if HPLC equilibrium is attained at each percentage of mobile phase during such measurements, a linear relationship between percent mobile phase and log k' or log $(t - t_0)$ should be apparent. Yamana *et al.* did demonstrate a linear dependence of log k' on the percentage of methanol used for elution (3). However, despite lower error and a better correlation using the extrapolated intercept at 0% methanol, they opted to use data measured at 30% methanol for procedural uniformity. Unfortunately, they failed to consider fully the significance of linearity. Deviations from linearity suggest a departure from HPLC equilibrium caused by changing interactions between the chemical and either the mobile or stationary phase. Although linearity of such plots over a limited solvent range can not prove that HPLC equilibrium is attained, curvilinearity is contrary evidence.

Unlike most procedures, ALPM utilizes the extrapolated linear intercept from computer-assisted log $(t - t_0)$ *versus* solvent composition measurements. As such, ALPM not only makes many more measurements, but in doing so better establishes equilibrium HPLC behavior for the agent being

0022-3549/84/1100-1623$01.00/0