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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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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

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Table I—Acid–Base Dependency of Certain ALPM Results with Methanol as the Organic Cosolvent [a]

Compound	Obs.	n_0	Rate, mL/min	Column Length, cm	Column Serial Number	Condition[b] Solvent Percentage	Oven Temp., °C	Solvent Percentage Low	Solvent Percentage High	n	r	C_{cf}[c]	ALPM log P	log P Range
Acetanilide	1	12	2	10	1516	1	35	15	50	12	0.9999	+0.00	+1.56 ± 0.01	+1.16 to +1.36
	2	19	2	10	1516	3	35	10	50	14	0.9998	+0.00	+1.18 ± 0.01	+1.16 to +1.36
	3	9	2	10	7172	3	32	15	55	9	0.9993	+0.09	+1.17 ± 0.03	+1.16 to +1.36
	4	8	2	10	9412	1	37	25	50	7	0.9995	+0.00	+1.66 ± 0.05	+1.16 to +1.36
	5	8	2	10	9412	1	37	30	50	5	0.9996	+0.00	+1.65 ± 0.08	+1.16 to +1.36
	6	8	2	10	9412	3	34	20	55	8	0.9998	+0.00	+1.19 ± 0.02	+1.16 to +1.36
	7	7	2	10	9412	4	37	30	50	5	0.9997	+0.00	+1.67 ± 0.07	+1.16 to +1.36
Benzene	8	17	2	10	1516	1	35	40	60	9	0.9998	+0.00	+2.15 ± 0.03	+1.56 to +2.15
	9	22	2	10	1516	3	35	30	60	10	0.9992	+0.00	+2.01 ± 0.04	+1.56 to + 2.15
	10	16	2	10	7172	3	35	35	60	9	0.9999	+0.09	+2.06 ± 0.02	+1.56 to +2.15
	11	14	3	10	1361	3	35	35	60	12	0.9996	+0.10	+1.93 ± 0.02	+1.56 to +2.15
Iodobenzene	12	13	3	1	0101	3	35	30	50	9	0.9999	−0.10	+3.29 ± 0.03	+3.25
	13	6	3	3	0001	3	35	25	50	6	0.9996	+0.00	+3.27 ± 0.06	+3.26
	14	6	4	3	0000	1	37	30	50	5	0.9997	+0.00	+3.48 ± 0.08	+3.25

[a] See Ref. 1. [b] Key: (1) 0.004 M trifluoroacetic acid; (2) 0.015 M triethylamine; (3) 0.035 M triethylamine; (4) 0.004 M acetic acid. [c] Column correction factor.

measured. The logarithm of the corrected retention volume, log V_{cr}, is converted by linear regression to the ALPM log P(o/w) (1). This paper describes the nature of this linearity and the ALPM linear extrapolation process. Several interesting discrepancies between ALPM and shake-flask log P(o/w) values are reported and discussed, and the ALPM-measured log P(o/w) values for certain phenylated, methylated, and halogenated benzenes are examined. This paper also compares equilibrium ALPM results with shake-flask and calculated log P(o/w) values used by Veith *et al.* to estimate the *in vivo* concentration process of chemicals in fish and by Helmer *et al.* to estimate the binding of chemicals to bovine serum albumin (4, 5).

EXPERIMENTAL SECTION

Definitions of terms, the equations for the calculation of ALPM log P(o/w), and a description of instrumentation, columns, conditions, and procedures are reported in the preceding paper (1).

RESULTS

Figure 1 illustrates that HPLC equilibrium [*i.e.*, linearity of log $(t - t_0)$ *versus* percent methanol] depends on the compound and not necessarily on the mechanical aspects of measurement. For instance, acetanilide, like the majority of compounds, gives a linear relationship between log $(t - t_0)$ and methanol percentage. Unlike acetanilide, but when run simultaneously, benzene seemingly interacts with the mobile phase, as demonstrated by the concave curvilinearity apparent at low percentages of methanol (Fig. 1). This increasingly rapid elution by water is pH independent; measurement using 0.004 M trifluoroacetic acid also affords a concave curvilinearity[1]. The benzene curve from ~60 to 35% MeOH is linear (*i.e.*, statistically, this region is not curved). Between these percentages, HPLC equilibrium is evidently maintained[2]. Extrapolation of the linear segment to 0% methanol and correction for conditions of measurement, as reported in the preceding paper for three separate determinations using 0.035 M triethylamine, indicates that the log P(o/w) for benzene is 2.00 ± 0.06 (*SD*); the relatively high standard deviation probably reflects the error in intercept measurement caused by curvilinearity. Although measurement using 0.004 M trifluoroacetic acid affords a concave curvilinearity, it yields a log P(o/w) of 2.15 ± 0.03 (*SE*), which is statistically identical to the benzene shake-flask value of 2.13. Regardless of the pH difference, either benzene curvilinear (*i.e.*, quadratic) intercept affords a log P(o/w) value substantially lower than that measured by extrapolation of the linear segment. This supports the midrange linear extrapolation approach.

In contrast with benzene, methylisobutylxanthine increasingly interacts with the stationary phase (indicated by the convex curvature in Fig. 1), but the cause for this phenomenon is not clear. Evaluation of this convex relationship is complicated by the unavailability of shake-flask results for methylisobutylxanthine. Future studies may be directed toward understanding these apparent disequilibria. For now, the results indicate that measurement of log $(t - t_0)$, particularly at a single, low alcoholic percentage (as is commonly practiced), could introduce substantial measurement error if HPLC equilibrium (*i.e.*, linearity) is not attained. This may have had considerable bearing on previous attempts to measure log P(o/w) by HPLC methods, regardless of column adsorbent.

While acids or bases can best be determined under acidic or basic conditions, respectively, ALPM measurement of neutral compounds can be performed in either acid or base (1). The data in Table I indicate that the extrapolated intercepts and log P(o/w) results for neutral compounds can be statistically the same (*e.g.*, iodobenzene) or they can differ (*e.g.*, benzene and acetanilide) depending on the measurement pH. These data are grouped primarily by compound name and secondarily by column serial number, but the results indicate column independence.

Figure 2 graphically illustrates results of ALPM log P(o/w) determinations of several benzene derivatives with phenyl, methyl, fluoro, chloro, and bromo substituents. Individual data for these compounds are listed in Table II. Figure 2 demonstrates that substituent additivity is generally invalid. Each substituent group appears to afford a concave relationship, although insufficient data are available for the fluoro and phenyl substituents to justify more than a linear

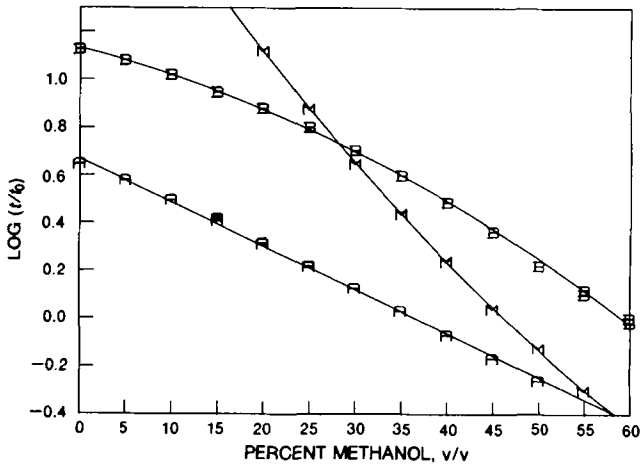


Figure 1—*Linearity of log* $(t - t_0)$ *data. Log* $(t - t_0)$ versus *percent methanol plots for acetanilide (A) and benzene (B) run simultaneously using 0.035 M triethylamine indicate that curvature depends on the compound. Methylisobutylxanthine (M) measured using 0.004 M trifluoroacetic acid gives a curvature opposite to that of benzene.*

[1] Unpublished results.

[2] Generally in ALPM, log $(t - t_0)$ values for methanol percentages of <25–30% are rarely measured. Not only do such measurements substantially increase the determination time, but the data often show greater statistical scatter (*e.g.*, Fig. 1, acetanilide between 8 and 15% methanol) or curvature (*e.g.*, Fig. 1, benzene and methylisobutylxanthine). Either condition requires that these points ultimately be excluded from the linear (*i.e.*, equilibrium) extrapolation. The results in Fig. 1 have been reproduced using several columns. Although not shown, high methanol percentages could also lead to curvature of log $(t - t_0)$. To maintain the greatest accuracy when measuring compounds with high partition coefficients, alcohol percentages of >60–65% should be used cautiously, and preferably with several points at and below 50% alcohol. Actually, use of these high percentages should be restricted to extremely high log P(o/w) compounds for which the lower values are difficult to obtain even on short columns. The difference $(t - t_0)$ should also exceed 0.3 min for greatest accuracy, although this empirical value actually depends on the accuracy of the elution-time measurements.

Table II—Partition Coefficient Summary for Various Substituted Benzenes

Compound	Obs.	ALPM log P	log P Range	Best[a]
Benzene	1	+2.01 ± 0.04	+1.56 to +2.15	+1.99
	2	+2.06 ± 0.02	+1.56 to +2.15	+1.99
	3	+1.93 ± 0.02	+1.56 to +2.15	+1.99
Biphenyl	4	+4.11 ± 0.11	+3.16 to +4.17	+4.09
	5	+4.13 ± 0.07	+3.16 to +4.17	+4.09
Bromobenzene	6	+3.02 ± 0.01	+2.99	+2.99
	7	+3.01 ± 0.02	+2.99	+2.99
	8	+3.06 ± 0.04	+2.99	+2.99
	9	+3.01 ± 0.06	+2.99	+2.99
Chlorobenzene	10	+2.79 ± 0.09	+2.18 to +2.84	+2.84
	11	+2.84 ± 0.06	+2.18 to +2.84	+2.84
	12	+2.81 ± 0.08	+2.18 to +2.84	+2.84
1,2-Dibromobenzene	13	+3.77 ± 0.06	+3.64	+3.64
	14	+3.68 ± 0.12	+3.64	+3.64
	15	+3.78 ± 0.02	+3.64	+3.64
1,2-Dichlorobenzene	16	+3.56 ± 0.07	+3.37 to +3.40	+3.40
1,3-Dichlorobenzene	17	+3.57 ± 0.04	+3.24 to +3.44	+3.44
1,4-Dichlorobenzene	18	+3.37 ± 0.05	+3.37 to +3.38	+3.37
Fluorobenzene	19	+2.14 ± 0.07	+2.27	+2.27
	20	+2.20 ± 0.04	+2.27	+2.27
	21	+2.22 ± 0.05	+2.27	+2.27
	22	+2.20 ± 0.07	+2.27	+2.27
Hexabromobenzene	23	+6.15 ± 0.14	—	—
	24	+6.07 ± 0.22	—	—
Hexachlorobenzene	25	+5.70 ± 0.17	+5.75	+5.75
	26	+5.79 ± 0.08	+5.75	+5.75
Hexafluorobenzene	27	+2.80 ± 0.09	+2.22	+2.22
Hexamethylbenzene	28	+5.00 ± 0.04	+4.31	+4.31
Mesitylene	29	+3.86 ± 0.03	+3.42	+3.42
	30	+3.87 ± 0.02	+3.42	+3.42
	31	+3.86 ± 0.02	+3.42	+3.42
	32	+3.87 ± 0.02	+3.42	+3.42
	33	+3.94 ± 0.01	+3.42	+3.42
Pentachlorobenzene	34	+5.21 ± 0.08	+4.94	+4.94
	35	+5.11 ± 0.06	+4.94	+4.94
	36	+5.21 ± 0.15	+4.94	+4.94
Pentamethylbenzene	37	+4.57 ± 0.02	—	—
	38	+4.55 ± 0.03	—	—
4-Terphenyl	39	+6.03 ± 0.07	—	—
	40	+5.88 ± 0.17	—	—
1,2,4,5-Tetrabromobenzene	41	+5.25 ± 0.05	—	—
1,2,3,5-Tetrachlorobenzene	42	+4.61 ± 0.04	+4.46	+4.46
	43	+4.73 ± 0.02	+4.46	+4.46
1,2,4,5-Tetramethylbenzene	44	+4.24 ± 0.02	—	—
Toluene	45	+2.70 ± 0.04	+2.11 to +2.80	+2.69
	46	+2.72 ± 0.02	+2.11 to +2.80	2.69
	47	+2.66 ± 0.09	+2.11 to +2.80	+2.69
1,3,5-Tribromobenzene	48	+4.60 ± 0.05	—	—
	49	+4.63 ± 0.05	—	—
1,3,5-Trichlorobenzene	50	+4.18 ± 0.03	—	—
1,2,4-Trifluorobenzene	51	+2.52 ± 0.07	—	—
2-Xylene	52	+3.18 ± 0.02	+2.77 to +3.12	+3.12
3-Xylene	53	+3.28 ± 0.01	+3.20	+3.20
4-Xylene	54	+3.29 ± 0.01	+3.15	+3.15

[a] Single literature value closest to ALPM log P.

fit. Table III lists ALPM-derived substituent constants, obtained by two different procedures, and shake-flask literature values (6). If the 0.035 M triethylamine value for benzene is used in the substituent constant calculation, the ALPM-derived constants are higher than those obtained by the octanol/water shake-flask method. These values closely resemble those obtained with the "hyperdiscriminating" solvents benzene or chloroform (7). If the monosubstituted benzene is used instead of benzene for the substituent constant calculation, the ALPM-derived value is lower and is closer to the shake-flask result. However, an average of these ALPM-derived values affords results close to the shake-flask substituent constant.

DISCUSSION

As a result of the potentially high error when using a single capacity factor measurement, many workers have begun to use the logarithm of the extrapolated intercept at 100% aqueous solvent in correlation work. For example, Butte *et al.* extrapolated data measured from 75–90% methanol to 0% methanol in their studies of various phenolic compounds eluting from an 10-μm octadecylsilane LiChrosorb RP-18 column (8). Bieganowska extrapolated values from 60–80% methanol (obtained using a 10-μm RP-18 column) in her investigation of the elution properties of *N*-phenylamides of benzoylacetic acid (9). In more extensive studies, Hafkenscheid and Tomlinson have examined 32 simple, but diverse, compounds over the range from 30 to

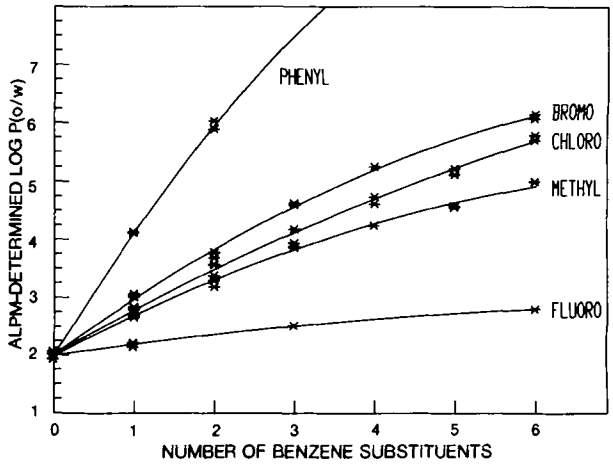


Figure 2—*ALPM log P(o/w)* versus *the benzene substituent number. This plot of ALPM log P(o/w) for various substituted benzenes* versus *the number of groups on the benzene ring reveals that strict substituent additivity is not the rule.*

90% methanol and extrapolated the RP-18 elution results to zero, generally obtaining a correlation coefficient of >0.999 (10). Braumann and Grimme used an extrapolated 0% methanol result obtained from 55 to 80% methanol with pyridazinones eluting over an RP-18 column (11). Close examination of the latter study reveals that correlation coefficients between 0.995 and 0.999 were often obtained, in contrast to the 0.999 minimum for ALPM (1), suggesting that curvature was perhaps unrecognized. Hanai and Hubert used acetonitrile/water from ≥30 to 90% in their study of urinary aromatic acids (12).

Questions arise as to the proper choice of solvent, solvent percentage range, and the extent of log $(t - t_0)$ *versus* percent solvent linearity for obtaining these extrapolated results. In this regard, an understanding of the way these factors can influence the log $(t - t_0)$ *versus* percent solvent relationship is pertinent. Acetonitrile, but not the alcoholic solvents, gave a curved plot of log $(t - t_0)$ as ordinate *versus* the percent of nonaqueous solvent for ALPM work with 1-(3-furyl)-4-methylpentan-1-one (1). The present study reveals, however, that curved plots can be obtained even in methanol. In their detailed studies, Schoenmakers *et al.* examined the elution behavior of 32 compounds from an RP-18 column using methanol, acetonitrile, or tetrahydrofuran; they also found plots of solvent fraction *versus* the logarithm of the capacity factor (as ordinate) were often curved, irrespective of the solvent (13). Although methanol afforded the greatest separation potential, it required such a long time for elution of highly nonpolar agents that it was useless. While other researchers have used higher methanol percentages to solve this problem, ALPM utilizes different column lengths for the less polar agents.

Hanai and Hubert, in their studies using an octadecylsilica column, found that curvature can occur at either high or low acetonitrile levels (12). Results obtained using methanol (Fig. 1) reveal that curvature can be either concave or convex, depending on the compound. A comparison of RP-8 ALPM findings for specific compounds with the results of Schoenmakers *et al.* using RP-18 columns reveals that compounds need not have the same concave or convex curvature; thus, curvature is also column dependent. Overall, HPLC equilibrium can depend on the organic solvent(s), the chemical, and/or the column, among other factors. Indeed, so many factors become important that one wonders if these relationships are ever really linear. In this regard, Schoenmakers *et al.* have theorized that these relationships should actually be qua-

Table III—Aromatic Substituent Constants[a]

Substituent	ALPM Constant: Mono-substituted[b]	ALPM Constant: Di-substituted[c]	ALPM Constant: Mean[d]	Shake-Flask Constant[e]
Phenyl	2.12	1.84	1.98	1.96
Methyl	0.69	0.56	0.63	0.56
Fluoro	0.19	—	—	0.14
Chloro	0.81	0.69	0.75	0.71
Bromo	1.03	0.72	0.88	0.86

[a] All ALPM values determined using 0.035 M triethylamine (1). [b] ALPM constant computed from the difference between ALPM values for the log P(o/w) of the monosubstituted benzene and benzene. [c] ALPM constant computed from the difference between ALPM values for the log P(o/w) of the disubstituted benzene and that of the monosubstituted benzene. [d] Average of mono- and disubstituted values. [e] Derived from octanol/water data (6).

Table IV—"Bioconcentration Factor" (Log BCF) Predicted from Various Log P(o/w) Measurements [a]

	Measured log BCF	Calculated log BCF		
		Other HPLC	ALPM	Calculated
Acenaphthene	2.58	3.18 (4.49)	2.83 (4.02)	3.15 (4.45)
Butyl benzyl phthalate	2.89[b]	3.38 (4.75)	3.64 (5.09)	—
2-Chlorophenol	2.33	0.40 (0.83)	1.53 (2.32)	1.42 (2.17)
1,2-Dichlorobenzene	1.95	2.62 (3.75)	2.47 (3.56)	2.47 (3.55)
1,3-Dichlorobenzene	1.82[b]	2.77 (3.95)	2.48 (3.57)	2.47 (3.55)
1,4-Dichlorobenzene	1.78	2.64 (3.78)	2.33 (3.37)	2.47 (3.55)
Diethyl phthalate	2.07	1.80 (2.67)	2.02 (2.96)	—
Dimethyl phthalate	1.76	—	1.19 (1.87)	0.96 (1.56)
2,4-Dimethylphenol	2.18[b]	1.28 (1.99)	1.64 (2.46)	1.70 (2.54)
N-Nitrosodiphenylamine	2.34[b]	2.47 (3.55)	2.19 (3.18)	—
1,2,3,4-Tetrachlorobenzene	3.26	3.57 (5.00)	3.32 (4.67)	3.55 (4.97)
Pentachlorobenzene	3.53	3.79 (5.29)	3.69 (5.16)	4.09 (5.68)[c]

[a] Numbers in parentheses are log P(o/w) values used to calculate log BCF by the formula of Veith *et al.* (4): log BCF = 0.76 × [log P(o/w)] − 0.23. [b] Measured log BCF was better approximated using "Other HPLC" or "Calculated" values than by "ALPM" log P(o/w) values. [c] Calculated from values determined by this study.

dratic (13). While it is difficult to explain why results are so often linear, it is clear that equilibrium (*i.e.*, state of linearity) must now be considered in HPLC methods for hydrophobicity determination, if more meaningful data are to be obtained.

Unfortunately, it is difficult to know the status of such equilibria without a time-consuming measurement of all significant log $(t - t_0)$ results at incremental percentages from 0 to 100% solvent. Although ALPM software can achieve this, once these data are obtained, they are often of little use[2]. If they are linear, only a few points would be needed, and the time required could be greatly reduced. If they are curved, it may be unclear at which end or over which region the curvature occurs. The only answer is to understand the real cause of this curvilinear behavior. However, the use by ALPM of statistically valid log $(t - t_0)$ *versus* 55–35% methanol percentage data with linearity (or curvilinearity) testing, as for benzene, seems to afford the fastest and, by covering the range of maximum solvent independence, the best assurance of the actual equilibrium condition.

HPLC-determined hydrophobicity is generally considered to be a composite of partition and absorption processes (14). If absorption events could be eliminated from HPLC measurements, then data from the HPLC partition process could be compared with that from the shake-flask method to discern discrepancies. However, absorption processes probably cannot be prevented using octadecylsilane columns because of a structured methanol layer on the column packing surface (14). ALPM utilizes C_8 or octylsilylated columns, which are less likely to bind methanol. In this regard, the high correlations of ALPM data with the shake-flask results over such a wide range, as reported in the first paper (1) and in Table IV herein, suggest absorption processes are of minimal importance. As a result, unexplainable differences between ALPM and shake-flask log P(o/w) results should be rare. However, discrepancies warrant attention. In general, they could signify an absorption process with the ALPM partition column, another ALPM anomaly, or perhaps even shake-flask irregularities. ALPM discrepancies could indicate how the processes unique to individual chemicals alter simple partitioning phenomena. While such deviations could have considerable relevance to predicting biological activities, they affect only that isolated value. On the other hand, shake-flask irregularities could also identify improperly computed substituent constants and errors in calculated values.

One inconsistency between the ALPM and shake-flask log P(o/w) values occurs for benzene, a primary standard for the calculation of log P(o/w). ALPM measurement using the established 0.035 M triethylamine conditions does not give the reproducible and accepted shake-flask value of 2.13, but affords a mean of 2.00 ± 0.06 for three determinations. ALPM measurement in 0.004 M trifluoroacetic acid yields 2.15 ± 0.03, which is statistically the same as the shake-flask result. The data indicate a possible pH dependency of log P(o/w); however, no significant variation of shake-flask log P(o/w) with pH is evident with benzene[3].

ALPM estimates of log P(o/w) reflect the ratio of chemical on the stationary phase (SP) to that in the mobile phase (MP) (Eq. 3). If the ALPM result has the same value as the shake-flask approach, pure partitioning would be indicated. If not, the ALPM result can be evaluated to determine which changes would give the altered ratio (1):

$$\log P(o/w)_{ALPM} = m \times \log ([X]_{SP}/[X]_{MP}) + b \qquad \text{(Eq. 3)}$$

where $[X]_{SP}/[X]_{MP} = V_{ccr}$.

Assuming the ALPM acid result and the reproducible 2.13 literature shake-flask log P(o/w) for benzene are correct, the lower ALPM value in base suggests that benzene is eluting earlier in base than in acid. This could result from a reduced interaction between benzene and the stationary phase or an increased interaction between benzene and the mobile phase. Four facts argue against the former possibility and support the latter: (*a*) excellent correlations are obtained under basic conditions for many agents having higher or lower log P(o/w) values; (*b*) benzene is chemically unlikely to combine with column functionalities; (*c*) it has no substituents to hinder column interactions; (*d*) the log $(t - t_0)$ *versus* percent methanol plot is curved regardless of pH for benzene, but not for acetanilide. The well-known benzene-water azeotrope also supports occurrence of such an interaction.

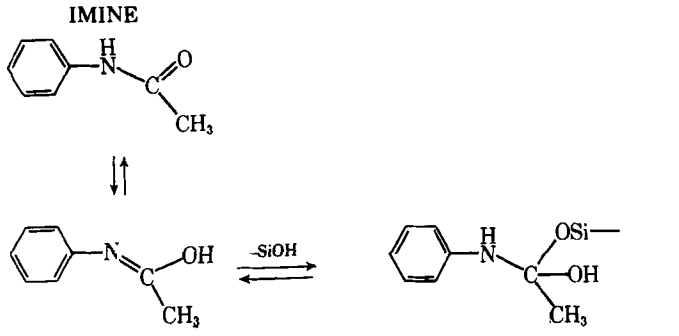
The lower base log P(o/w) observation has some generality. Base-determined ALPM values for the smaller, more water-soluble, heterocyclic compounds pyrrole, furan, and thiophene, are similarly lower than their shake-flask counterparts (Table V, obs. 21, 45, and 46) (6, 15, 16). Increasing compound size appears to diminish the differences between ALPM and the shake-flask results. The 2.20 average ALPM value for fluorobenzene is lower than the 2.27 shake-flask result. Fluorobenzene, like benzene, affords a curvilinear relationship between log $(t - t_0)$ and percent methanol. However, no curvilinearity or deviation was apparent with chlorobenzene (Table II, obs. 10–12 and 19–22). Similarly, the ALPM value for the larger aromatics naphthalene and anthracene are statistically the same as their shake-flask values.

These data could support the existence of a hydrogen-bonded solvation shell about the smaller aromatic agent. Masking of lipophilic aromatics by a hydrophilic shell could impede their column binding; it would, however, decrease as the size of the aromatic chemical increases. Knowledge of whether the complete log $(t - t_0)$ *versus* percent methanol relationship for these heteroaromatic agents is curvilinear would be useful in evaluating this proposal, but their weak UV absorption (when compared with baseline triethylamine-methanol solutions) has precluded obtaining that information. The lower ALPM value for benzene in base, as opposed to acid, could arise from stronger hydrogen bonding caused by a greater electron density on the hydroxyl ion. Such a solvation shell, if formed, could also reduce penetration of these smaller π-excessive aromatic agents through lipid-like biomembranes, suggesting that the ALPM result may be more relevant than the shake-flask result in predicting biological properties. However, no comparative biological data are presently available to support that suggestion.

Different values for ALPM and shake-flask results can occur even when a linear relationship is evident, regardless of measurement pH. The 1.17 value for acetanilide obtained in base using ALPM is consistent with the pH-independent literature shake-flask result[4], requiring the higher ALPM value obtained in acid to be explained (Table I). The possibility of a fast trifluoroacetylation reaction was discounted by substitution of acetic acid (Table I, obs. 7). Since the acid plot of log $(t - t_0)$ *versus* percent methanol is also linear, the higher value can only be explained by increased column binding by acetanilide under acid conditions. In this regard, reversible Michael addition of a silanol residue to a multisubstituted and benzene-stabilized imine by the process in Scheme I is proposed. While occurrence of this base-catalyzed process in dilute acid may seem strange, the acid could represent a compromise between the needed tautomerization and sorbant-imine-catalyzed Michael reaction. The Michael reaction is a highly reversible process (17); its occurrence would statistically delay departure of the compound from the column. The present data are insufficient to determine that this reaction actually occurs on the column. Theoretically, it would be prevented if no free, unalkylated silanol residues were present. Future studies using spherical, extensively capped packing materials could conceivably eliminate this process. If such a chemical event could also occur with nucleophiles in membranes, the ALPM determi-

[3] J. E. Garst and A. Leo, unpublished results.

[4] Albert Leo, personal communication.



Scheme I

Table V—Partition Coefficients of Miscellaneous Compounds

Compound	Obs.	ALPM log P	log P Range	Best[a]
Acridine	1	+3.31 ± 0.03	+3.40	+3.40
4-Aminopyridine	2	+0.33 ± 0.04	+0.28	+0.28
Anisole	3	+1.97 ± 0.08	+2.04 to +2.11	+2.04
Anthracene	4	+4.66 ± 0.09	+4.45	+4.45
	5	+4.67 ± 0.09	+4.45	+4.45
2-Benzofuroic acid	6	+2.51 ± 0.04	—	—
3-Bromoquinoline	7	+2.90 ± 0.02	+3.03	+3.03
3-Bromothiophene	8	+2.59 ± 0.03	—	—
Caffeine	9	+0.65 ± 0.03	−0.07 to +0.01	−0.07
	10	+0.61 ± 0.04	−0.07 to +0.01	−0.07
3-Cyanopyridine	11	+0.54 ± 0.01	+0.36	+0.36
1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT)	12	+6.40 ± 0.11	+3.98 to +6.19	+6.19
4,4′-Dibromobiphenyl	13	+5.66 ± 0.07	—	—
2,3-Dibromothiophene	14	+3.53 ± 0.06	—	—
Diethylstilbestrol	15	+5.46 ± 0.11	+5.07	+5.07
1,2-Dihydroxybenzene	16	+0.95 ± 0.06	+0.84 to +1.01	+0.92
	17	+0.90 ± 0.11	+0.84 to +1.01	+0.92
2,4-Dinitrophenol	18	+1.79 ± 0.06	+1.50 to +1.54	+1.54
Phenylphosphono-thioic acid *O*-ethyl *O*-*p*-nitrophenyl ester (EPN)	19	+5.34 ± 0.08	+3.49	+3.49
Ferulic acid	20	+2.37 ± 0.03	—	—
Furan	21	+1.13 ± 0.02	+1.34	+1.34
Hexabromobiphenyl	22	+7.80 ± 0.15	—	—
4-Hydroxycinnamic acid	23	+2.20 ± 0.05	—	—
Methylisobutyl-xanthine	24	+2.29 ± 0.08	—	—
	25	+2.24 ± 0.08	—	—
3-Methylindole	26	+2.50 ± 0.09	+2.60	+2.60
3-Methylpyridine	27	+1.18 ± 0.01	+1.20 to +1.24	+1.20
α-Naphthoflavone	28	+4.82 ± 0.12	—	—
β-Naphthoflavone	29	+4.94 ± 0.08	—	—
1-Naphthoic acid	30	+3.36 ± 0.02	+3.10	+3.10
	31	+3.43 ± 0.02	+3.10	+3.10
α-Naphthylthiourea	32	+1.66 ± 0.02	—	—
4-(4-Nitrobenzyl)-pyridine	33	+2.88 ± 0.04	—	—
4-Nitrophenol	34	+1.96 ± 0.08	+1.38 to +2.08	+1.91
Ochratoxin A	35	+4.74 ± 0.11	—	—
[2.2]Paracyclophane	36	+4.47 ± 0.04	+2.33	+2.33
Phenol	37	+1.53 ± 0.09	+1.46 to +1.51	+1.49
Phenylacetic acid	38	+1.95 ± 0.04	+1.41 to +1.51	+1.51
	39	+1.96 ± 0.05	+1.41 to +1.51	+1.51
4-Phenylphenol	40	+3.63 ± 0.03	+3.20	+3.20
	41	+3.54 ± 0.03	+3.20	+3.20
Phenylthiourea	42	+0.77 ± 0.03	+0.73	+0.73
Progesterone	43	+5.01 ± 0.17	+3.70 to +3.87	+3.87
	44	+4.89 ± 0.10	+3.70 to +3.87	+3.87
Pyrrole	45	+0.62 ± 0.04	+0.75	+0.75
Thiophene	46	+1.74 ± 0.02	+1.81	+1.81
4-Toluic acid	47	+2.67 ± 0.02	+2.27	+2.27
	48	+2.66 ± 0.03	+2.27	+2.27
Trifluoromethyl-benzene	49	+3.37 ± 0.02	+2.79 to +3.01	+3.01
	50	+3.35 ± 0.02	+2.79 to +3.01	+3.01
2,4,6-Trinitrophenol	51	+1.46 ± 0.02	+2.03	+2.03
α-Tropolone	52	+1.54 ± 0.04	+0.53 to +0.75	+0.75

[a] Single literature value closest to ALPM log P.

Table VI—Prediction of Log Bovine Serum Albumin Binding (1/*C*)

Compound	log 1/*C*	Calculated log 1/*C*[a] Literature Value	Calculated log 1/*C*[a] ALPM
Phenol	3.32	3.40 (1.46)	3.46 (1.54)
3-Fluorophenol	3.86	3.75 (1.93)	3.71 (1.88)
4-Fluorophenol	3.52	3.63 (1.77)	3.66 (1.81)
3-Chlorophenol	4.30	4.18 (2.50)	4.08 (2.37)
4-Chlorophenol	4.00	4.10 (2.39)	4.12 (2.42)
4-Bromophenol	4.22	4.25 (2.59)	4.20 (2.53)
4-Iodophenol	4.40	4.49 (2.91)	4.38 (2.76)
4-Methylphenol	3.70	3.76 (1.94)	3.79 (1.98)
3-Ethylphenol	4.22	4.10 (2.40)	4.21 (2.54)
3-Trifluoromethylphenol	4.52	4.51 (2.95)	4.49 (2.91)
3-Cyanophenol	3.26	3.22 (1.22)	3.68 (1.84)[c]
3-Hydroxyphenol	3.15	2.90 (0.80)	2.93 (0.84)
3-Methoxyphenol	3.54	3.49 (1.58)	3.57 (1.69)
4-Methoxyphenol	3.40	3.31 (1.34)	3.45 (1.53)
3-Nitrobenzyl alcohol	2.94	3.18 (1.17)	3.34 (1.38)[c]
4-Methoxybenzyl alcohol	2.94	3.13 (1.10)	3.25 (1.27)[c]
Benzonitrile	3.23	3.47 (1.56)	3.53 (1.63)
Acetophenone	3.31	3.49 (1.58)	3.56 (1.67)
Nitrobenzene	3.58	3.69 (1.85)	3.68 (1.83)
4-Bromoacetanilide	4.00	3.94 (2.18)[b]	4.05 (2.33)
4-Nitroanisole	4.00	3.82 (2.03)[b]	3.97 (2.22)
4-Chloronitrobenzene	4.07	4.10 (2.39)	3.97 (2.23)
2,4-Dichloronitrobenzene	4.59	4.50 (2.93)[b]	4.39 (2.78)
Naphthalene	4.91	4.83 (3.37)	4.90 (3.47)
Azobenzene	5.29	5.17 (3.82)	5.61 (4.40)[c]
Anisole	4.00	3.89 (2.11)	3.80 (1.99)
3-Fluoroaniline	3.09	3.28 (1.30)	3.35 (1.41)
4-Chloroaniline	3.68	3.68 (1.83)[b]	3.71 (1.87)
4-Methoxyaniline	2.92	2.89 (0.78)[b]	1.94 (0.85)
4-Bromoaniline	4.06	3.83 (2.03)[b]	3.86 (2.07)
4-Methylaniline	3.30	3.34 (1.39)	3.38 (1.44)
1-Naphthylamine	3.94	3.98 (2.23)[b]	3.87 (2.09)
Indole	4.07	3.91 (2.14)	3.92 (2.15)
Thymol	4.66	4.78 (3.30)	5.00 (3.59)

[a] Determined using the regression constants reported by Helmer *et al.* (5), the value of log P(o/w) given in parentheses, and: log (1/*C*) = 0.751 × log P(o/w) + 2.301. [b] Calculated log P(o/w) used by Helmer *et al.* (5). [c] ALPM log P(o/w) and Helmer *et al.* (5) data differ by more than ±3 *SD* based on the shake-flask error (*i.e.*, 6.13 ± 2.76%) reported in the preceding paper (1).

nation in acid might more adequately reflect biological activities than would either the pure solvent/water partition measurement or the new, improved end-capped, partition column packings.

Several other compounds have also given ALPM estimates of log P(o/w) that differ from the shake-flask result. In principle, if triethylamine (pK_a = 11.01) forms an ion-pair with silanol residues, as suggested in the preceding paper (1), an amine of comparable basicity would do the same, delaying its elution and raising its apparent log P(o/w) value. Amines of lower basicity would not be affected. Thus, little difference between shake-flask and ALPM values has been found for diphenylamine (pK_a = 0.79), 3-bromoquinoline (pK_a = 2.69), 4-bromoaniline (pK_a = 3.86), pyridine (pK_a = 5.25), and acridine (pK_a = 5.58) (1). Although the ALPM value of 4-aminopyridine (pK_a = 9.11) appears unaffected, phenethylamine (pK_a = 9.84) affords an ALPM value of 2.84, ~0.4 units higher than the shake-flask result (6). This discrepancy for phenethylamine could prove advantageous in studies of new column packings. The absence of unalkylated silanol groups on a particular packing should theoretically result in no deviation for this agent and provide an excellent experimental tool to examine silica gel alkylation.

Two other compounds studied appear inconsistent with shake-flask data. Both the reproducible shake-flask method and the octanol-saturated, kieselguhr-packed column of Mirrlees *et al.* indicate that the log P(o/w) for caffeine ranges from −0.07 to 0.01 (2, 6). These values differ considerably from the reproducible ALPM value of ~0.63 (Table V). A base-related explanation for the high ALPM value of caffeine is unlikely; however, literature shake-flask data also suggest inconsistencies in the xanthine series. While the −0.07 value of Anderson, cited by Hansch and Leo, is consistent with the findings of others, his report of −0.02 for theophylline and −0.80 for theobromine, two dimethylated xanthines which should have similar values, complicate evaluation of this matter (6).

ALPM also does not afford a result similar to the shake-flask literature for α-tropolone (Table V). Although some enhanced column-binding process cannot be discounted, the 1.54 ± 0.04 ALPM value is close to the 1.45 shake-flask value of the similar *p*-hydroxyacetophenone (6). Likewise, a value of 1.61, estimated from that of catechol (Table V) and the ALPM substituent constant for a methyl group, is closer to the 1.54 ALPM value than to the

Table VII—Comparison of Results in Table VI

Source	Regression Equations	No. of Compounds
Helmer *et al.* (5)	$\log (1/C) = 0.751\ (\pm 0.070) \times \log P(o/w) + 2.301\ (\pm 0.150)$	42
Helmer *et al.* (5)	$\log (1/C) = 0.785\ (\pm 0.064) \times \log P(o/w) + 2.234\ (\pm 0.138)$	34
ALPM	$\log (1/C) = 0.752\ (\pm 0.092) \times \log P(o/w) + 2.246\ (\pm 0.204)$	34

	Helmer *et al.* (5)	Helmer *et al.* (5)	ALPM
r	0.960	0.976	0.949
SE of estimate	0.159	0.130	0.189
n	42	34	34
Calculated log $(1/C)$ values differ <0.1:		21 of 34 compounds	
Observed log $(1/C)$ values closer to Helmer *et al.* (5):		7 of 13 compounds	
Observed log $(1/C)$ values closer to ALPM:		6 of 13 compounds	

0.53–0.75 range reportedly obtained by shake-flask (6). α-Tropolone shows no evidence of curvilinearity in its log $(t - t_0)$ *versus* percent methanol plot. Furthermore, this deviation occurs at a log P(o/w) value where other compounds give virtually identical shake-flask and ALPM results (Table V). The fact that α-hydroxy ketones in general, and α-tropolone specifically, readily hydrate and dimerize may be relevant to these discrepancies (18). Perhaps biological properties may be better predicted by the higher ALPM result, especially if the higher ALPM log P(o/w) value reflects partitioning of a stable dimer or unhydrated species.

In the course of these determinations various substituted benzenes were examined (Fig. 2). Concave curvature of their ALPM log P(o/w) values continues from 0 to 6 substituents seemingly without regard to the electron-donating or electron-withdrawing character of the substituent. Figure 2 also indicates that values for the various fluorobenzenes deviate the least from linearity when compared to methyl, phenyl, chloro, or bromo substituents. This near linearity for the smaller fluorine groups suggests that spatial rather than electronic factors influence ALPM log P(o/w). Other correlation parameters, such as molar refractivity, are known to have a major spatial component (6). Such factors may also explain results with [2.2]paracyclophane, which has a reported shake-flask log P(o/w) of 2.33 (6). ALPM yields the much higher value of 4.47 (Table V), yet even this value is significantly lower than that expected based on double the 3.29 ALPM value for *p*-xylene (Table II).

Since ALPM affords a continuous, wide-range estimation of log P(o/w) by a column-binding method and since considerable shake-flask data also indicate that these groups are not truly additive (6), perhaps strict additivity is simply fallacious. This would suggest clear limitations on the calculation of log P(o/w) values using various additivity schemes. However, even calculations should afford minimal error when one or two benzene substituents are present. In this case, ALPM often gives values very close to the calculated value (using benzene = 2.13). ALPM gives 3.56 and 3.57 for both the 1,2- and 1,3-dichlorobenzenes, respectively, but only 3.37 for 1,4-dichlorobenzene. The shake-flask procedure gives 3.40 and 3.44 for the 1,2- and 1,3-dichlorobenzenes, respectively, but 3.37 for 1,4-dichlorobenzene. Veith *et al.* reported 3.55 as the calculated value for all the dichlorobenzenes (4). Since ALPM gave that value for 1,2- and 1,3-dichlorobenzenes, but afforded the noticeably lower 3.37 shake-flask value for the *para* isomer, perhaps ALPM can detect real differences between isomers which are not evident in calculations. Occasionally, ALPM better supports the calculated value for compounds with sterically noninteracting substituents. For instance, the shake-flask log P(o/w) of mesitylene is 3.42 (6) and the calculated value is 3.81 (6), yet ALPM yields 3.87.

Attempts to calculate log P(o/w) values using various additivity schemes may lead to inaccurate values for interacting, multiply substituted, and high log P(o/w) compounds; these compounds have log P(o/w) values which are otherwise difficult to measure directly and, in part, prompted development of the additivity concept. Thus, ALPM gives interesting differences between shake-flask and/or calculated values for such compounds as 1,2,3,4-tetrachlorobenzene or pentachlorobenzene (Table IV), and hexamethylbenzene [ALPM, 5.00; shake-flask, 4.31 (6); calculated, 5.49 (6)]. These findings and interesting discrepancies for phenylphosphothioic acid *O*-ethyl *O*-*p*-nitrophenyl ester (EPN) and progesterone in Table V could suggest that the best way to determine log P(o/w) is ALPM measurement.

One clear comparison of method is the prediction of biological data. In this regard, ALPM log P(o/w) values seem to afford equal or better predictions. Table IV compares ALPM log P(o/w) data with those obtained from calculated values and another HPLC method in predicting the measured *in vivo* concentration process of various compounds in bluegill sunfish (4, 19). For 8 of 12 compounds having sufficient UV absorption to allow evaluation with the current detector system, ALPM measurement gave equal or better approximation of the "bioconcentration" factor (as log BCF) than did either the calculated or HPLC-determined values reported by Veith *et al.* (4, 19). Since, *in vivo* measurement of the concentration process may cost from $3000 to $10,000 (depending on the need for radiolabeled agents), this represents a significant savings (4).

Log P(o/w) data for 34 compounds, having sufficient availability and UV absorption to enable ALPM measurement, were compared with the combination of shake-flask and calculated log P(o/w) values reported by Helmer *et al.* with regard to prediction of bovine serum albumin binding (Table VI) (5). Correlation coefficients, standard errors, and other measures of similarity are shown in Table VII. Several major discrepancies occur between shake-flask and ALPM values. Although Helmer *et al.* measured a value of 1.22 (5) and Hansch and Leo cite 1.70 as the shake-flask value of 3-cyanophenol (6), ALPM measured an even higher 1.84. Calculations would suggest a value near 0.89; however, electron-withdrawing groups tend to greatly increase this value. Thus, the ALPM value for 3-cyanophenol is similar to both the ALPM and shake-flask result for 4-nitrophenol (Table V). In view of the measurement error in the binding term, log $(1/C)$, and the wide range of literature shake-flask log P(o/w) values cited for these compounds, these methods are judged indistinguishable.

In summary, any direct HPLC method is quicker and can ensure measurement of the correct component better than shake-flask techniques. But only ALPM, which measures considerable elution data, can easily offer considerable insight into whether equilibrium is obtained between the mobile and stationary HPLC phases. Examination of benzene suggests that if linearity (*i.e.*, equilibrium) is not attained, altered physical interactions between the chemical and the mobile or stationary phases may be responsible for deviations between ALPM and the shake-flask result. If linearity is attained, acetanilide data suggest that altered column-binding interactions may more satisfactorily explain deviations between these measurement methods. Full understanding of chemical-mobile phase and chemical-stationary phase interactions is required to elucidate chemical factors which could change the partition coefficient apparent in biological situations. In addition to providing a computer-operated method to expedite this data acquisition, ALPM estimation of log P(o/w) and of biological activities can offer improved or equal accuracy, a major cost reduction, and a speed greater than existing HPLC and shake-flask log P(o/w) determinations. In the few cases where ALPM and shake-flask results differ, more research is necessary to establish the exact nature of the discrepancy, with biological data affording the ultimate tool to determine the most suitable parameter. Future ALPM developments should further enhance accuracy by enabling the simultaneous measurement of the void volume marker, the compound of interest, and internal log P(o/w) standard, all under computer control.

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Generic Tolbutamide Tablet Dissolution: Intralot and Interlot Variation

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Received August 8, 1983, from the **School of Pharmacy, Oregon State University, Corvallis, OR 97331* and the [‡] *Medical Bioavailability Unit, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication October 26, 1983.

Abstract □ Dissolution profiles for 62 lots of tolbutamide tablets from six manufacturers have been characterized using the USP paddle-stirrer apparatus. Results of paddle-stirrer dissolution for percent drug dissolved at 10, 20, and 30 min correlated well ($r^2 = 0.7444$) with results from the USP rotating-basket test for 39 lots of tolbutamide. Interlot and intralot variability in tolbutamide dissolution was highly dependent on the manufacturer. For one product, the intralot range (for six paddle-stirred tablets) of percent drug dissolved after 30 min was 50–68% while the maximum interlot range for mean dissolution was 58–104%. One lot failed to meet both the rotating-basket and the paddle-stirrer dissolution specifications. Tablet response to aging at 60, 75, and 98% relative humidity over time was also highly manufacturer specific. The innovator's product repeatedly dissolved well when fresh or aged at all humidities. Dissolution from some generic tablets was dramatically depressed by humidity aging, even after only 3 d. Pretreatment of tablets with simulated gastric fluid modified the dissolution profile of one poorly dissolving lot of tablets. Results indicate that manufacturing quality control is highly variable among tolbutamide tablets.

Keyphrases □ Tolbutamide—dissolution, intra- and interlot variation, effect of humidity aging □ Dissolution studies—tolbutamide, intra- and interlot variation, effect of humidity aging

Tolbutamide tablets must meet United States Pharmacopeia (USP) dissolution requirements which are established because ". . . in many cases it is possible to correlate dissolution rates with biological availability of the active ingredient" (1). Dissolution testing is also recognized (2) as useful for quality control purposes.

Tolbutamide exhibits only limited solubility and the United States Food and Drug Administration (FDA) recognizes that tolbutamide products are prone to bioavailability problems. Currently, the FDA lists 12 manufacturers' products as "therapeutically equivalent to each other" (3). Large variations in lot-to-lot dissolution of tolbutamide products indicate variations in quality and possible variations in bioavailability since low tolbutamide bioavailability is sometimes related to poor tolbutamide tablet dissolution (4–6). If such variations occur for different lots of products which have been ruled "therapeutically equivalent" by the FDA, then satisfaction of current FDA regulations may not assure lot-to-lot equivalence of products on the FDA list.

Previous work has shown extensive lot-to-lot variation in dissolution of tolbutamide tablets for six lots of one manufacturer (7), while there was uniformity for the originator's product. Others have demonstrated that humidity "aging" of tolbutamide tablets differentially affected dissolution for two different brands of products (8). Also, the physiological exposure to acid of ingested tablets is not mimicked by the current USP dissolution test although exposure of tolbutamide tablets to gastric acid has been reported to be necessary for disintegration and dissolution of some formulations (9).

Therefore, the purposes of this study were: (*a*) to evaluate the dissolution characteristics of several fresh lots of tolbutamide listed as therapeutically equivalent by the FDA; (*b*) to evaluate many of the same lots after humidity "aging" of the tablets; (*c*) to determine the effect of tablet exposure to gastric acid on tolbutamide dissolution for both fresh and humidity-aged tablets.

EXPERIMENTAL SECTION

Products were obtained commercially, stored in tightly closed, opaque containers in the dark at room temperature for 1–3 months, and tested using the USP rotating-basket (10) or paddle-stirrer dissolution test (1). Samples (3 mL) were collected with a continuous flow (set at 5–10 mL/min) eight-channel peristaltic pump[1] fitted with stainless steel 20–30 μm in-line filters. Samples were collected for 6 tablets for 10, 20, 30, and 45 min for the rotating-basket test (150 rpm) and at 10, 20, and 30 min for the paddle-stirrer test (75 rpm) in order to establish a dissolution *versus* time profile (rather than single time point dissolution values). All samples were replaced with temperature-equilibrated dissolution medium.

Filtered samples were diluted, the UV absorbance was measured at 226 nm (1), and the concentration of tolbutamide was calculated based on a seven point standard curve prepared the same day as unknowns were collected. Standard curves were generated by preparing known concentrations of either USP reference standard or company-provided tolbutamide. The UV spectra from these sources were superimposable and the standard curves considered equivalent as the null hypothesis of equal slopes and intercepts could not be rejected ($\alpha = 0.05$). Standard curves were fit with parabolic regression and had coefficients of variation of <4%. The average inversely estimated percent

[1] Gilson minipuls 2, eight channel peristaltic pump; Gilson Medical Electronics, Middleton, Wis.

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