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High-throughput determination of octanol/water partition coefficients using a *shake-flask* method and novel two-phase solvent system

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ABSTRACT

A high-throughput method for determining the octanol/water partition coefficient ($P_{o/w}$) of a large variety of compounds exhibiting a wide range in hydrophobicity was established. The method combines a simple *shake-flask* method with a novel two-phase solvent system comprising an acetonitrile–phosphate buffer (0.1 M, pH 7.4)—1-octanol (25:25:4, v/v/v; AN system). The AN system partition coefficients (K_{AN}) of 51 standard compounds for which $\log P_{o/w}$ (at pH 7.4; $\log D$) values had been reported were determined by single two-phase partitioning in test tubes, followed by measurement of the solute concentration in both phases using an automatic flow injection-ultraviolet detection system. The $\log K_{AN}$ values were closely related to reported $\log D$ values, and the relationship could be expressed by the following linear regression equation: $\log D = 2.8630 \log K_{AN} - 0.1497 (n = 51)$. The relationship reveals that $\log D$ values (+8 to –8) for a large variety of highly hydrophobic and/or hydrophilic compounds can be estimated indirectly from the narrow range of $\log K_{AN}$ values (+3 to –3) determined using the present method. Furthermore, $\log K_{AN}$ values for highly polar compounds for which no $\log D$ values have been reported, such as amino acids, peptides, proteins, nucleosides, and nucleotides, can be estimated using the present method. The wide-ranging $\log D$ values (+5.9 to –7.5) of these molecules were estimated for the first time from their $\log K_{AN}$ values and the above regression equation.

1. Introduction

The logarithm of the octanol/water partition coefficient, $\log P_{o/w}$, provides information regarding the physicochemical properties of drugs and industrial chemicals. The $\log P_{o/w}$ is the most widely employed descriptor of the hydrophobicity, lipophilicity, and quantitative structure-activity relationships of biological, pharmaceutical, and environmentally relevant compounds [1–4]. Over the past few decades, numerous methods for experimental determination or estimation of $\log P_{o/w}$ values have been developed, the most common of which is the *shake-flask* method, in which an analyte is simply partitioned between 1-octanol and water (or aqueous buffer) phases in a flask or test tube [1]. After partitioning, the analyte concentration in both phases is quantified by off-line UV–vis absorption or other spectrometric detection or via on-line flow-injection analysis (FIA) [5,6], high-performance liquid chromatography (HPLC) [7–10], or gas chromatography [7,11]. Although the traditional *shake-flask* method is reliable for direct $\log P_{o/w}$ determination, it is time-consuming, labor intensive, and requires a relatively large amount of pure analyte. More effective methods improved by incorporating modern techniques/apparatus, such as solid-phase microextraction [11], liquid–liquid microextraction [7,8], or 96-well plate formats [9,10], have recently been developed. As a striking example, Dohta et al. [10] established a high-throughput system for $\log D$ ($\log P_{o/w}$ at pH 7.4) screening in a 96-well plate using a water-plug aspiration/injection method combined with HPLC–mass spectrometry. Using this system, $\log D$ values spanning the range +5.9 to –1.9 were determined for 25 drugs.

In contrast to *shake-flask* methods, liquid–liquid countercurrent chromatography (CCC) using octanol and water as stationary and/or mobile phases is an ideal method for high-throughput direct $\log P_{o/w}$ determination. CCC permits automatic calculation of the $P_{o/w}$ from the retention time (more accurately, the retention volume) of a solute in a CCC chromatogram, without the need for manual handling of two-phase partitions, dilution, or quantifi-

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Table 1
Experimentally determined log K_{AN} and literature-reported log D values of 51 standard compounds.

Compound	Measured value				Literature value
	K_{AN}	SD (n = 4)	RSD %	log K_{AN}	log D[a]
Amiodarone	84.385	2.12	2.5	1.926	6.1
Tolnaftate	74.496	8.278	11.1	1.87	5.4
Clotrimazole	37.266	1.787	4.8	1.571	5.2
Diethylstilbestrol	44.712	0.604	1.4	1.65	5.07
Bifonazole	41.398	0.597	1.4	1.617	4.77
Loratadine	39.732	1.175	3	1.599	4.4
Estradiol	16.595	0.838	5	1.22	4.01
Ketoconazole	20.667	0.484	2.3	1.315	3.83
3,5-Dichlorophenol	9.593	0.511	5.3	0.982	3.68
Chlorpromazine	8.356	0.133	1.6	0.922	3.38
Naphthalene	25.035	1.431	5.7	1.399	3.37
Thioridazine	12.867	0.74	5.8	1.109	3.34
Testosterone	12.008	0.344	2.9	1.079	3.29
Nifedipine	19.087	0.293	1.5	1.281	3.17
Clozapine	12.891	0.283	2.2	1.11	3.13
3-Bromoquinoline	13.924	0.509	3.7	1.144	3.03
Diazepam	14.018	0.577	4.1	1.146	2.79
Trazodone hydrochrolide	12.925	0.254	2	1.111	2.54
3-Chlorophenol	11.711	0.156	1.3	1.069	2.5
Imipramine	4.779	0.079	1.7	0.679	2.4
Carbamazepine	6.106	0.072	1.2	0.786	2.19
Diltiazem	11.228	0.361	3.2	1.05	2.06
Quinoline	6.015	0.255	4.2	0.779	2.03
Dexamethasone	6.733	0.047	0.7	0.828	1.83
Lidocaine	12.615	0.338	2.7	1.101	1.71
Desipramine	2.1	0.006	0.3	0.322	1.28
Propranolol	1.805	0.039	2.1	0.257	1.26
Chloramphenicol	5.553	0.117	2.1	0.744	1.14
Alprenolol	1.694	0.032	1.9	0.229	0.97
Trimethoprim	2.208	0.051	2.3	0.344	0.63
Clonidine hydrochloride	3.523	0.042	1.2	0.547	0.62
Fluconazole	2.207	0.049	2.2	0.344	0.5
Antipyrine	2.104	0.038	1.8	0.323	0.38
Cimetidine	1.443	0.066	4.6	0.159	0.35
Pentoxifylline	2.259	0.041	1.8	0.354	0.29
Nitrofurazone	2.345	0.021	0.9	0.37	0.23
Metronidazole	1.662	0.01	0.6	0.221	−0.02
Caffeine	1.31	0.014	1	0.117	−0.07
Metoprolol	0.747	0.02	2.7	−0.126	−0.16
Thiamphenicol	2.357	0.041	1.7	0.372	−0.27
Ranitidine hydrochloride	0.875	0.012	1.3	−0.058	−0.29
Acebutolol	0.694	0.01	1.4	−0.159	−0.29
Nizatidine	1.209	0.007	0.6	0.082	−0.52
Atropine	0.488	0.017	3.4	−0.312	−0.55
Allopurinol	0.517	0.003	0.5	−0.286	−0.44
Pirenzepine	0.529	0.02	3.8	−0.276	−0.61
Disopyramide	0.922	0.003	0.3	−0.035	−0.66
Tiapride	0.879	0.012	1.4	−0.056	−0.90
Procainamide	0.423	0.008	1.9	−0.374	−0.91
Terbutaline sulfate	0.21	0.005	2.5	−0.677	−1.35
Sotalol	0.254	0.003	1.1	−0.595	−1.35

[a] All log D values were quoted from Ref. [19].

cation of the solute concentration [12–15]. However, CCC is too time-consuming for determining log $P_{o/w}$ values greater than +3 and/or less than −3.

Because 1-octanol is a malodorous and highly viscous solvent, other systems for high-throughput indirect log $P_{o/w}$ estimation have been developed utilizing techniques such as reversed-phase (RP) HPLC [16–21], RP–thin layer chromatography (TLC) [2,22–24], micelle electrokinetic chromatography (MEKC) [25,26], and microemulsion electrokinetic chromatography (MEEKC) [27–30]. In these chromatographic methods, linear regression is used to correlate both the logarithm of the retention factor (log k) and log $P_{o/w}$ parameters, although the methods are typically used with relatively simple molecules or those within a homologous series. Furthermore, these methods can be used to estimate positive log $P_{o/w}$ values (up to +6) of hydrophobic compounds, but they are not suitable for estimating negative log $P_{o/w}$ values of very hydrophilic compounds, which are poorly retained on the typical alkyl stationary phases used with these chromatographic systems (e.g., octadecyl (C_{18}) groups, sodium dodecyl (C_{12}) sulfate (SDS) micelles, or SDS microemulsions).

To the best of our knowledge, the maximum and minimum log $P_{o/w}$ values reported in the literature are approximately +6 (for coronene [30], DDT [3,17,26], and amiodarone [19]) and approximately −2 (for L-tyrosine [26] and acyclovir [30]), respectively. However, there are many compounds important in the pharmaceutical and life sciences research fields for which the log $P_{o/w}$ values may be higher or lower than the currently reported maximum and minimum values. In particular, the log $P_{o/w}$ values of highly polar bioactive compounds such as peptides, proteins, nucleotides, and related drugs have yet to be elucidated.

In this report, we describe a new high-throughput method for determining the log $P_{o/w}$ values of an extended range of hydrophobic and/or hydrophilic compounds. The overall method combines a simple *shake-flask* method with a novel two-phase solvent system.