

PARTITION COEFFICIENT

Log D

The partition coefficient between water or buffer and n-octanol or cyclohexane is the most widely used measure of chemical compound lipophilicity. Lipophilicity is a major structural factor governing both pharmacokinetics and pharmacodynamics of drugs.

Cerep has developed high-throughput microplate assays for the determination of this important physicochemical parameter.

- ▶ Partition coefficient (Log D, n-octanol/PBS, pH 7.4)
- ▶ Partition coefficient (Log D, cyclohexane/PBS, pH 7.4)

DEFINITION OF PARTITION COEFFICIENT

The partition coefficient of a chemical compound provides a thermodynamic measure of its hydrophilicity-lipophilicity balance. In the laboratory, a small amount of compound is added to a system of two essentially immiscible liquids consisting of an aqueous phase (water or buffer) and an organic phase. Once the compound has fully equilibrated with the two solvents, the partition coefficient can be calculated by dividing the concentration of compound present in the organic phase by the concentration of compound present in the aqueous phase and applying the logarithm. For example, if the concentration of a compound in the organic phase were tenfold higher than in the aqueous phase, the partition coefficient would be 1.

WHAT SIGNIFICANCE DOES THE PARTITION COEFFICIENT HAVE FOR DRUG DISCOVERY?

Modern screening technology and the recent combinatorial chemistry trend toward generation of more lipophilic molecules has resulted in HTS hits that are generally more lipophilic than marketed drugs or compounds currently undergoing clinical evaluation. High lipophilicity often goes with poor aqueous solubility. This can bring with it many challenges often making development of a seemingly promising drug candidate very difficult.

Lipophilicity is also a major structural factor that influences the pharmacokinetic and pharmacodynamic behavior of compounds. Partitioning within a biological system and biological activity are governed by recognition forces that are, among others, defined by hydrophobic interactions. Strong hydrophobic interactions can result in non specific binding with proteins (target and non-target) in the aqueous biological environment. A hydrophobic drug molecule has a thermodynamic tendency to reduce the surface area exposed to water. Hydrophobic compounds will tend to bind to hydrophobic surfaces through Van der Waals bonds. For compounds with extracellular or cell-surface targets it appears prudent to restrict lipophilicity and thereby avoid easy diffusion across biological membranes and into cells, cellular compartments, and the CNS. On the other hand, a certain degree of lipophilicity is required to allow a drug to enter cellular organelles or to cross the blood-brain barrier. Having lipophilicity information available at the early discovery stage therefore is critical information that can help chemists design new molecules and enables biologists to better interpret screening results.

Lipophilicity is a key factor determining *in vivo* behavior of drugs

- ▶ **Pharmacokinetics:**
 - . Permeation of physiological membranes (absorption, distribution)
 - . Plasma protein binding
 - . Volume of distribution
- ▶ **Pharmacodynamics:**
 - . Target recognition
 - . Target affinity
 - . Target specificity

WHY USE N-OCTANOL?

The properties of n-octanol are thought to resemble those of lipid bilayer membranes. It has therefore been suggested that distribution of chemicals into n-octanol simulates, to a certain extent, their ability to passively diffuse across biological membranes.

WHY USE CYCLOHEXANE?

Lipophilicity is to some degree dependent on the hydrogen bonding ability of the analyte and solvent used. Unlike, n-octanol, cyclohexane does not possess hydrogen bonding characteristics and for this reason water-cyclohexane distribution is believed to more closely model blood-brain barrier partitioning behavior. More specifically data sets have been obtained which show that the difference between log D_{oct} and log D_{cyc} shows a highly significant inverse correlation with the logarithms of brain/blood concentrations (Young *et al*, *J. Med. Chem.*, 1988, 31, 656-671).

LOG P, LOG_{KOW}, LOG D, CLOG P, MLOG

There are many symbols in use for partition coefficient and each one represents a different measure. The experimentally determined partition coefficient using pH conditions pro-

Frequently used methods for the determination of partition coefficient

- ▶ **Direct method:** Shake-flask (compound distribution between n-octanol and buffer)
- ▶ **Indirect methods:** Chromatographic, Electrometric titration
- ▶ **Computational methods:** Clog P, Mlog P

▶ PARTITION COEFFICIENT - Log D

viding for uncharged compound in the aqueous and octanol phases is called log P (for partition) or log K_{OW} . If a buffer of a given pH is used, the resulting partition coefficient is called log D (for distribution) or log D pH 7.4 if buffer with pH 7.4 was used. Compounds may be partially ionized in this case. Rather than using aqueous phases that completely suppress ionization, log P values are often determined by extrapolation from log D values. Clog P and Mlog P refer to partition coefficients that are calculated using two different mathematical algorithms and structural information about the molecule in question. The ranges of log P found with these mathematical models are much wider than the ranges typically found with experimental log P or log D determinations, which makes a comparison between mathematically and experimentally derived values difficult.

■ CEREP'S PARTITION COEFFICIENT ASSAYS: LOG D USING MINIATURIZED SHAKE-FLASK

Cerep has two assays available for the measurement of partition coefficient in the range from approximately -0.5 to 4.5. Both assays are based on a miniaturized shake-flask procedure. Buffer (Dulbecco's PBS, pH 7.4) is generally used as the aqueous phase. In this case, the correct symbol for the resulting partition coefficient is log D pH 7.4. The two assays differ simply in the organic phase (n-octanol or cyclohexane) used for partitioning. Both assays start with compound dissolved in DMSO. Final DMSO and compound concentrations (1% and 100 μ M respectively) during the organic-buffer partitioning are very low to avoid bias on the partitioning. The amount of compound in the buffer phase is determined by HPLC with photodiode array detection. The amount of compound in the organic phase is calculated by subtraction of the amount of compound in buffer from the total amount of compound, which is determined from a calibration sample. Since the organic layer is not directly analyzed, Cerep's partition coefficient assay does not distinguish solubilization in the organic layer from precipitation. Both assays were designed to deliver high-throughput results with minimal use of compound. Test compounds need to contain a chromophore for photodiode array detection. The procedures are based on 96-well plate technology and take advantage of modern laboratory automation.

▶ SMALL AMOUNT OF COMPOUND REQUIRED

The test can be run with as little as 10 nmol of compound. 1 mg of pre-weighed compound suffices.

▶ RAPID TURNAROUND TIME

The results are delivered within two weeks upon receipt of the compounds at Cerep's testing site. Data are made available on line as soon as they are validated.

■ CUSTOMIZATION

Buffers other than PBS can be accommodated.

■ QUALITY CONTROL

Each assay includes eight reference compounds as internal controls.

These compounds were selected for molecular diversity and have log D values across the entire range of the assay. Only assays whose reference compounds fall within specified ranges pass quality inspection.

Log D _{oct} assay	Mean	Acceptable range	Log D _{cyc} assay	Mean	Acceptable range
metoprolol tartrate	-0.36	-0.58 to -0.25	metoprolol tartrate	-1.91	-3.00 to -1.00
rifampicin	1.28	1.00 to 1.40	rifampicin	-1.17	-2.80 to 0.65
ketoconazole	3.48	3.27 to 3.64	ketoconazole	-0.09	-0.30 to 0.20
phenytoin	2.32	2.00 to 2.37	phenytoin	-0.27	-1.20 to 0.50
haloperidol	2.76	2.47 to 2.84	haloperidol	0.45	0.25 to 1.00
simvastatin	4.49	4.25 to 4.95	simvastatin	1.80	1.50 to 2.20
diethylstilbestrol	> 4.5	> 4.5	diethylstilbestrol	1.71	1.35 to 2.10
tamoxifen	> 4.5	> 4.5	tamoxifen	> 4.0	> 4.0

Partition coefficients of various drugs as determined with Cerep's assays. Compounds were distributed between Dulbecco's PBS, pH 7.40, and either n-octanol or cyclohexane. Results are expressed as the log D pH 7.40 ▼

Compound	Log D _{oct}	Log D _{cyc}	Compound	Log D _{oct}	Log D _{cyc}	Compound	Log D _{oct}	Log D _{cyc}
ajmaline	1.09	-0.83	estrone	3.69	1.59	nifedipine	3.57	-0.10
albuterol	-1.72	0.77	ethacrynic acid	1.16	-2.04	norethindrone	3.15	1.40
amityriptaline	2.77	1.70	flecainide	1.08	-0.87	paclitaxel	3.62	2.91
atenolol	-1.72	0.53	flufenamic acid	2.10	-0.51	phenytoin	2.28	-0.14
caffeine	-0.10	-1.23	flunarizine	> 4.7	4.70	pimozide	4.50	2.44
captopril	-0.81	-0.57	fluoxetine	2.08	1.06	progesterone	3.84	1.80
carbamazepine	1.65	-0.72	furosemide	-0.89	-1.77	propranolol	1.05	-0.96
cefotaxime	-1.77	-4.42	griseofulvin	2.07	0.88	reserpine	3.73	4.30
chloramphenicol	1.08	-2.58	haloperidol	2.81	0.32	strychnine	0.93	0.41
chlorpheniramine	1.25	0.48	hydrocortisone	1.52	-0.87	sulfaphenazole	0.10	-1.74
chlorpromazine	3.17	2.44	imipramine	2.31	1.68	sulfisoxazole	-1.46	-1.32
colchicine	1.10	-2.07	ketoconazole	3.48	-0.09	tamoxifen	4.51	4.60
dantrolene	1.53	1.74	labetolol	0.99	-1.53	terfenadine	4.05	1.51
desipramine	1.38	0.26	lidocaine	1.61	0.96	tetracycline	-1.28	-1.24
dexamethasone	1.95	-1.70	mexiletine	0.49	-0.62	thalidomide	0.64	-0.87
dextromethorphan	1.43	0.65	mianserin	3.26	2.30	tolbutamide	0.40	-1.26
diltiazem	2.01	0.62	minoxidil	0.88	-1.65	verapamil	2.41	1.12
disopyramide	-0.52	-0.60	nicardipine	3.62	1.96	warfarin	0.85	-1.31



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■ QUESTIONS OR CONCERNS?

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