

LogP dodecane-water - comparison with intrinsic permeability through membranes of 2% DOPC in dodecane

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Introduction

A number of pharmaceutical research laboratories have taken up the PAMPA technique since it was described by Kansy¹ in 1998. A widely used PAMPA methodology is based on permeation through an artificial membrane made by painting a 2% solution of dioleoylphosphatidylcholine (DOPC) in dodecane on to an inert porous support, and measuring the permeation across this membrane between two buffer solutions at the same pH – the so-called iso-pH PAMPA method. In this study, we used this method to determine intrinsic permeability, which we compared with logP measured in dodecane-water by the pH-metric method for 39 compounds. We noted a strong correlation between intrinsic permeability in the 2% DOPC in dodecane system and logP dodecane-water.

Experimental

63 ionisable compounds with good UV absorption (mainly drugs) were selected for measurement. One other criterion for selection was that the pK_a value that defined the neutral species fell within the pH range of the assay. Both PAMPA permeability and logP dodecane-water were successfully measured for 39 compounds. Problems encountered during measurement of the remaining compounds are listed in Table 1.

The effective permeability values were measured at 12 pHs between n 3 and 10 with 16-hour incubation times, using a pON Evolution system with pH mapping, Tecan Genesis robot and Molecular Devices SpectraMax UV plate reader. The pK_a values used in the conversion of effective to intrinsic permeability were obtained from the literature, or measured, either pH-metrically using Sirius GLpKa, or by pH/UV using the BPAS attachment.

LogP dodecane-water values were measured pH-metrically by GLpKa and Sirius RefinementPro 2 software. In this method, logP values are calculated from the shift in pK_a that occurs when a sample is titrated in a two-phase system. After sample preparation, all functions were automatic, including calculation of results and pH-lipophilicity profiles.

It is essential that samples are in solution throughout the measurement of dodecane-water logP by the pH-metric method. Measurements are made by titration in solutions between 10 and 20 mL volume. Since all samples are ionisable, the best way to ensure solution is to weigh the compound, add dodecane manually, and then start the titration at a pH where the sample will become ionised (low pH for bases, high pH for acids). If samples are not prepared in this way, they may precipitate at the water-dodecane interface.

Results

Results are listed in Table 1.

Values for P_e, the effective permeability for a solute with non-sink conditions (taking membrane retention of solute into account), were obtained from pONs Evolution software. Intrinsic permeability (P_o) is related to P_e as shown in our poster entitled **Practical Approaches to Measuring Intrinsic Permeability and an LFER Model of Intrinsic Permeability**. In essence, the pK_a(flux) and logP_u are varied systematically using the Solver function in Microsoft Excel to produce calculated logP_e values that fall on a "best-fit" curve of logP_e vs. pH. The pK_a(flux) value that produces the best fit is subtracted from the true aqueous pK_a and the difference is added to the optimised logP_u to produce a value for logP_o. The logP_o value and the true aqueous pK_a are then used to calculate values for logP_m. This method and equations are described elsewhere².

LogP dodecane-water values were calculated from the shifts in pK_a that occurred in dual-phase titrations. These shifts may be visualised by Bjerrum difference curves like the example shown in Figure 1 for the measurement of Tramadol. Because of the extreme lipophilic character of dodecane, no ion-pair partition was observed for any of the compounds studied.

Figure 2 shows a graph of intrinsic permeability (logP_o) plotted against logP dodecane-water for 39 compounds from Table 1.

Sample name	LogP dodecane-water	Intrinsic permeability (logPo)	Samples with PAMPA problems	Problem observed
Aspirin	-1.82	-5.57	Bepiridil	
4-nitrophenol	-2.22	-5.25	Miconazole	
Theophylline	-1.70	-6.77	Nitrofurantoin	
Desipramine	3.38	-0.83	Prazosin	Noisy data or poor solubility
Phenylacetic acid	-1.60	-5.03	Prochloraz	
Fluoxetine	-1.37	-5.19	Terfenadine	
Nitrozapine	-1.10	-5.08	Tryptamine	
3, 5-Dimethoxyphenol	-1.06	-4.12		
2, 4-Dichlorophenoxyacetic acid	-0.86	-4.38	Atropine	
4-Hydroxybenzoic acid	-0.75	-5.61	Ephedrine	Poor UV
Furosemide	-0.60	-4.94		
Eserine(Physostigmine)	-0.46	-4.62	Atenolol	
Metoprolol	-0.38	-4.11	Famotidine	
Aniline	-0.23	-3.36	Fenoterol	Nothing in acceptor well
Procaine	-0.67	-3.99	Moxonidine	
Warfarin	0.05	-2.90	Pindolol	
Carazolol	0.05	-3.99	Triamterene	
4-Propoxybenzoic acid	0.31	-3.55		
2-Naphthoic acid	0.72	-3.40		
Papaverine	0.75	-3.06		
Indomethacin	0.98	-3.54		
4-Phenylbutylamine	1.00	-2.13		
Lidocaine	1.00	-2.51		
Propranolol	1.75	-2.12		
Diltiazem	1.37	-2.67		
Flurbiprofen	1.49	-2.55		
Haloperidol	1.53	-2.15		
Tetracaine	1.54	-1.98		
Diclofenac	1.72	-2.58		
Tramadol	1.82	-1.49		
Ibuprofen	2.08	-2.46		
Verapamil	2.27	-1.54		
4-Fluorodeprenyl	2.64	-0.73		
Diphenhydramine	2.67	-0.94		
Penbutolol	3.06	0.30		
Fluoxetine	3.10	0.07		
Chlorpromazine	-4.37	0.94		
Quinine	-0.29	-4.14		
Phenazopyridine	0.42	-2.74		
Carbendazim		-4.82		
Chlorhalidon		-6.32		
Sulfacetamide		-6.68		
Fenoterol		-6.52		
Naproxen	0.53	-2.60		
2-Iodophenol	0.80	-2.34		
3-Chlorophenol	-0.31	-4.00		
Phenol	-1.28	-4.34		
Thymol	1.40	-2.55		

Table 1. Measured logP dodecane-water and Intrinsic permeability. All values measured at Sirius unless highlighted in colour. The graph in Figure 2 was plotted using data for the 39 compounds shown in bold.

Cells with green background denote published measured logP values in other alkalines.
4-Nitrophenol (cyclohexane), Aspirin, Theophylline (heptane)

Cell with blue background denotes logP measured at Sirius in cyclohexane-water

Cells with yellow backgrounds denote logP dodecane-water values supplied by Elizabeth Glyn, Organon UK

Cells with orange background denote compounds for which logP dodecane-water was not measured.

Permeability values in cells marked red were calculated using Absolv software, with an Abraham equation based on the PAMPA coefficients listed in table 2. These values were plotted on the graph, but not included in the linear fit. The last four compounds equilibrated in the PAMPA assays, i.e. the concentrations in the donor and acceptor wells were equal.

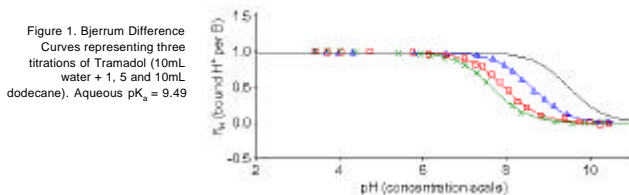


Figure 1. Bjerrum Difference Curves representing three titrations of Tramadol (10mL water + 1, 5 and 10mL dodecane). Aqueous pK_a = 9.49

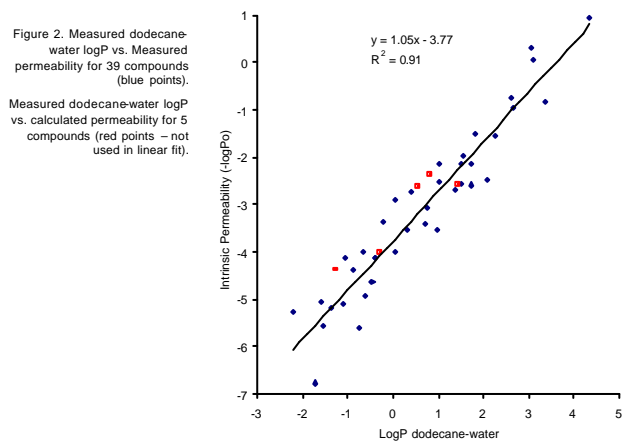


Figure 2. Measured dodecane-water logP vs. Measured permeability for 39 compounds (blue points). Measured dodecane-water logP vs. calculated permeability for 5 compounds (red points – not used in linear fit).

Table 2. Comparing coefficients of Abraham equations

	c	e	s	a	b	v
PAMPA permeability (40 solutes)	-4.26	1.15	-1.60	-1.68	-2.89	3.03
LogP cyclohexane/water (170 solutes) ⁷	0.16	0.78	-1.68	-3.74	-4.93	4.58
LogP hexadecane/water (370 solutes) ⁷	0.09	0.67	-1.62	-3.59	-2.89	3.03

Discussion

We observed a good correlation (slope of 1.05 and r² of 0.91) between measured logP dodecane-water values and permeability measured through membranes made from a 2% solution of DOPC in dodecane. While recent research has described PAMPA with membranes made from more concentrated lipid solutions³, a good deal of PAMPA work has been done using membranes made from 1 – 2% solutions of lipids in dodecane⁴.

Faller has reported a good correlation between measured logP hexadecane values and PAMPA permeability measured through membranes made from pure hexadecane on a filter support, as well as a good correlation between these values and human GI absorption for a set of (unidentified) compounds⁵.

Our results suggest that the membrane behaved as if it were made from pure alkane. The Abraham equation derived from our permeability values (as reported in poster entitled **Practical Approaches to Measuring Intrinsic Permeability and an LFER Model of Intrinsic Permeability**) is similar to equations published for alkane-water partitioning, as shown in Table 2.

Our PAMPA and alkane studies could not detect absorption of all compounds. Though they had good UV spectra, we could not detect 6 compounds in the acceptor wells, suggesting that their permeability was very low. The logP dodecane-water values for these compounds were also too low to measure. Nevertheless, all these compounds are marketed drugs, and published pharmacokinetic data suggests that they are well absorbed.

Conclusion

If the ratelimiting step in drug uptake by passive diffusion is the passage through the membrane's lipid core, as modelled by PAMPA studies using alkanes, then logP dodecane-water would appear to provide equivalent results. It's easy to measure logP pH-metrically for ionisable compounds, and the cost of instrumentation is relatively low.

References

- Kansy, M. Senner, F. Gubemator, K. Physicochemical high throughput screening: Parallel artificial membrane permeation assay in the description of passive absorption processes. *J Med Chem.* 1998 (41(7)) pp 1007-1010
- Huque, F T T, Box, K, Platts, J A, Comer, J. Profiling passive absorption through artificial membranes 2004. Submitted
- Bermejo, M, Audeof, A, Ruiz, A, Nalda, R, Ruell, J A, Tsinman, O, Gonzalez, I, Fernandez, C, Sanchez, G, Garrigues, T M, Merion, V. PAMPA - a drug absorption *in vitro* model. 7. Comparing Rat *in situ* Caco-2 and PAMPA permeability of fluorquinolones. 2004, submitted
- Audeof, A, Strafford, M, Block, E, Balogh, M P, Chambliss, W, Khan, I. Drug absorption *in vitro* model: filter-immobilised artificial membranes 2. Studies of the permeability properties of lactones in Piper Methysticum Forst. *European J Pharm. Sci.* 2001 (14) pp 271-280
- Zhu, C, Jiang, L, Chen, T M, Hwang, K K. A comparative study of artificial membrane permeability assay for high throughput profiling of drug absorption potential. *Eur. J. Med. Chem.* 2002 (37) pp 399-407
- Wohnsland, F, Faller, B. High-Throughput Permeability pH Profile and High-Throughput Alkan/Water logP with Artificial Membranes. *J. Med. Chem.* 2001 (44(6)) pp 923-930
- Abraham, M H, Chadha, H S, Whiting, G S, Mitchell, R C. Hydrogen-bonding. 32. An analysis of water-octanol and water-alkane partitioning and the delta-logP parameter of Seiler. *J Pharm Sci* 1994 (83(8)) pp 1085-1100
- Leo, A, Hansch, C, Elkins, D. Partition coefficients and their uses. *Chem. Rev.* 1971 (71) pp 525-616