Relative Partition Coefficients from Partition Functions: a Theoretical Approach to Drug Transport

Jonathan W. Essex, Christopher A. Reynolds, and W. Graham Richards*

Oxford Centre for Molecular Sciences and Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ, U.K.

The free-energy perturbation method has been applied to the calculation of the difference in partition coefficients (P) between methanol and ethanol partitioned between water and carbon tetrachloride; calculated and experimental values agree to within 0.06 log P units.
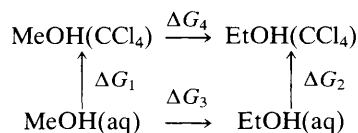
Partition functions can be employed to calculate partition coefficient differences. The importance arises from the utility of partition coefficients (P) for measuring transport properties of drugs as influenced by solubility in lipid membranes.[1—3] Many structure–activity relationships of biological activity are based on partition properties, notably in the work pioneered by Hansch[4] in which activity is correlated with the logarithm of the partition coefficient between water and octanol (log P). Usually, partition coefficients are experimental values or derived using empirical rules based on building molecules from fragments. We show that it is possible to calculate theoretical partition coefficient differences with a precision of 0.14 log P units using the free-energy perturbation method,[5—8] offering the possibility of both computing values for novel compounds in drug design and perhaps creating a theoretical model membrane which mimics biological reality more closely than octanol.

The choice of test system is governed by the need for reliable experimental data.[4] The system selected is methanol and ethanol partitioned between water and carbon tetrachloride, the latter being chosen as the co-solvent to ensure efficient sampling of configuration space in an accessible simulation time, although in principle any solvent could have been used. The partition coefficient (P) of an alcohol, ROH, is defined[2] in equation (1), where the species ROH is monomeric. Experimentally, the distribution coefficient is determined at a range of solute concentrations and an extrapolation to infinite dilution gives the partition coefficient.

$$\log P = \log([ROH]_{CCl_4}/[ROH]_{aq}) \qquad (1)$$

Considering the thermodynamic cycle shown in Scheme 1, the Gibbs free energies ΔG_1 and ΔG_2 determine the log P values for methanol (MeOH) and ethanol (EtOH) respectively, where the standard state corresponds to monomeric solute in solvent at 293.15 K. It would seem natural to calculate log P values by simulating the processes for ΔG_1 and

$$MeOH(CCl_4) \xrightarrow{\Delta G_4} EtOH(CCl_4)$$

$$\Big\uparrow \Delta G_1 \qquad \xrightarrow{\Delta G_3} \qquad \Big\uparrow \Delta G_2$$

$$MeOH(aq) \longrightarrow EtOH(aq)$$

Scheme 1

ΔG_2. However, such simulations would involve the removal of a solute from one solvent and its immersion in the second, a procedure which would take an inordinate time in a molecular dynamics simulation. However, a relative log P, as determined by $\Delta G_1 - \Delta G_2$, can be calculated given knowledge of ΔG_3 and ΔG_4, since $\Delta G_3 - \Delta G_4 = \Delta G_1 - \Delta G_2$. These Gibbs free energy differences, ΔG_3 and ΔG_4, can readily be obtained by means of the free-energy perturbation relationship[9] in equation (2), which gives the difference in free energy

$$\Delta G = -RT \ln <\exp(-H_{AB}/RT)>_A \qquad (2)$$

of two similar systems A and B. H_{AB} is the difference in the Hamiltonians of systems A and B and represents a perturbation on state A: $H_B = H_A + H_{AB}$. $< >_A$ indicates that an average is taken over the configurations of state A. Such configurations can be generated using molecular dynamics. The Hamiltonian is formulated classically as a sum of bond, angle, torsional, non-bonded repulsion–dispersion, and electrostatic terms. The advantage of the free-energy perturbation method is that, although derived from the relationship between the partition function and free energy, it avoids the explicit calculation of the partition function. Recent applications yielding encouraging results, both in terms of the reproducibility of the statistics and also in terms of the agreement with experiment, have been reported by a number of groups.[5—8,10,11] To ensure rapid convergence of equation (2) within a reasonable simulation time, ΔG must be less than approximately $2kT$. This condition is implemented by dividing the perturbation into a number of smaller simulations (windows). The Hamiltonian used in each simulation is defined with the aid of a coupling parameter λ, equation (3).

$$H_\lambda = \lambda H(MeOH) + (1 - \lambda)H(EtOH) \qquad (3)$$

During the forward simulation, λ is reduced from 1 to 0 in steps of 0.05 giving a total of 21 windows in the course of the entire simulation. Similarly, the backward simulation corresponds to the change EtOH → MeOH. Each system is equilibrated prior to data collection to allow sampling of dominant configurations. The free energy change for the perturbation of methanol into ethanol is then given by the sum of the free energy changes associated with each window.

The molecular dynamics simulations were performed using the AMBER[12] suite of programs. Approximately 500 TIP3P[13] water molecules and 188 carbon tetrachloride molecules were used in the simulations. The carbon tetrachloride parameters and charges used are those generated by McDonald[14] (model 2), since adopted by Bermejo.[15] The alcohol parameters were generated in a manner consistent with the AMBER program.[16,17] The electrostatic charges were derived so as to reproduce the quantum mechanically derived electrostatic potential which was calculated using the GAUSSIAN 86[18] and CHELP[19] programs. These calculations employed a 3–21G basis set.[20] Non-bonded cut-offs of 8 Å and 13 Å were used in the water and carbon tetrachloride simulations respectively. Both systems were equilibrated at constant temperature and pressure (1 atm, 293.15 K) using periodic boundary conditions

Table 1. A comparison of calculated and experimental differences in free energies and partition coefficients.

	ΔG_3/kJ mol^{-1}	ΔG_4/kJ mol^{-1}	$\Delta \log P^a$
Forward simulation	−0.561	−4.546	—
Backward simulation	−1.465	−4.630	—
Average[b]	−1.01 ± 0.68	−4.59 ± 0.39	−0.64 ± 0.14
Experiment[c]	0.28 ± 1.53		−0.70

[a] The calculated $\Delta \log P$ value is given by $2.303\Delta\log P = -(\Delta G_3 - \Delta G_4)/RT$, and the experimental value by $\Delta \log P = \log P(MeOH) - \log P(EtOH)$. [b] The error bounds are calculated from the standard deviations for the forward and backward simulations. [c] The error bars arise from a 5% uncertainty in the free energies of hydration of methanol and ethanol.[24]

for between 10 and 20 ps, a time step of 0.002 ps being used in conjunction with SHAKE.[21] The simulation conditions correspond to infinite dilution in the sense that the solute molecules do not interact with each other as in the experimental standard state. At each window, between 500 and 1500 steps of equilibration preceded 500 steps of data collection.

The results of the simulations are given in Table 1 along with a comparison of the theoretical and experimental values for $\Delta \log P$. A number of distinct experimental values of log $P(EtOH)$ are known[4] which differ considerably; the most reliable datum is that obtained most recently by Korenman *et al.*,[22] although no experimental error is quoted. As this is the only source for log $P(MeOH)$, the effect of any systematic errors present in the experimental results is minimized. The experimental determination was carried out at a range of concentrations and the resulting distribution coefficient was found to be concentration independent. It was therefore assumed that alcohol association did not occur, and so the distribution coefficient was equated to a partition coefficient. The calculated and experimental values of $\Delta \log P$ agree remarkably well. Moreover, the discrepancy is less than the standard deviation of results from the forward and backward simulations. The larger error on ΔG_3 compared with ΔG_4 is probably due to the greater complexity of water as a solvent compared with carbon tetrachloride, but the error is typical of values obtained by this method. As further justification as to the validity of these results, the calculated value of ΔG_3 lies within the error bounds of the experimental value,[23] the latter corresponding to a difference in the free energies of hydration of ethanol and methanol corrrected to 293.15 K using enthalpy data.[23] The authors[24] of the experimental work quote an error of 5% on their measurements, and we have assumed that this is propagated through to the determination of the free energies.

It must be stressed, however, that reliable results may only be obtained given the availability of suitable potentials. Moreover, for the purposes of the simulations, water and carbon tetrachloride are assumed to be completely immiscible. Although this is not, in fact, the case, we consider solvent miscibility to have a minimal effect on log P for this system, because mutual solubility is very small. We also assume that the alcohols do not promote mutual solubility, because it has been shown that the partition coefficient is independent of alcohol concentration. Indeed, our approach may give a more meaningful measure of solute partitioning than the experimental model of membranes.

The calculations are computationally intensive, and for this reason were performed on the Rutherford Appleton Labora-

tory CRAY X-MP. Our results clearly indicate that the accurate calculation of partition coefficients is both feasible and, indeed, desirable. We now propose to simulate lipid membrane systems with the aim of calculating partition coefficients which are directly relevant to biological activity, but which cannot be determined by experiment. Although here we have calculated differences in partition coefficients, the same technique could be used to provide absolute values if we were to take as our reference system a zero size, zero charge atom in the two solvents and then grow this point into a methanol molecule using perturbation methods.[25]

References

1 A. Gringauz, 'Drugs: how they act and why,' C. V. Mosby Company, St. Louis, 1978.
2 Y. C. Martin, 'Quantitative Drug Design: A Critical Introduction,' Marcel Dekker, Inc., New York, 1978.
3 M. S. Tute, *Adv. Drug Res.*, 1971, **6**, 1.
4 C. Hansch and A. Leo, 'Substituent Constants for Correlation Analysis in Chemistry and Biology,' Wiley-Interscience, New York, 1979.
5 P. Cieplak, U. C. Singh, and P. A. Kollman, *Int. J. Quantum Chem.*, 1987, **QBS14**, 65.
6 C. F. Wong and J. A. McCammon, *J. Am. Chem. Soc.*, 1986, **108**, 3830.
7 U. C. Singh, F. K. Brown, P. A. Bash, and P. A. Kollman, *J. Am. Chem. Soc.*, 1987, **109**, 1607.
8 S. N. Rao, U. C. Singh, P. A. Bash, and P. A. Kollman, *Nature*, 1987, **328**, 551.
9 R. W. Zwanzig, *J. Chem. Phys.*, 1954, **22**, 1420.
10 J. P. M. Postma, H. J. C. Berendsen, and J. R. Haak, *Faraday Symp. Chem.*, 1982, **17**, 55.
11 C. A. Reynolds, P. M. King, and W. G. Richards, *Nature*, 1988, **334**, 80.
12 U. C. Singh, P. K. Weiner, J. W. Caldwell, and P. A. Kollman, AMBER (Version 3.1), Department of Pharmaceutical Chemistry, University of California, San Francisco, 1988.
13 W. L. Jorgensen, J. Chandrasekhar, J. Madura, R. W. Impey, and M. L. Klein, *J. Chem. Phys.*, 1983, **79**, 926.
14 I. R. McDonald, D. G. Bounds, and M. L. Klein, *Mol. Phys.*, 1982, **45**, 521.
15 F. J. Bermejo, E. Enciso, J. Alonso, N. Garcia, and W. S. Howells, *Mol. Phys.*, 1988, **64**, 1169.
16 S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profeta, Jr., and P. Weiner, *J. Am. Chem. Soc.*, 1984, **106**, 765.
17 S. J. Weiner, P. A. Kollman, D. T. Nguyen, and D. A. Case, *J. Comput. Chem.*, 1986, **7**, 230.
18 M. Frisch, J. S. Binkley, H. B. Schlegel, K. Raghavachari, R. Martin, J. J. P. Stewart, F. Bobrowicz, D. Defrees, R. Seeger, R. Whiteside, D. Fox, E. Fluder, and J. A. Pople, Gaussian 86, release C, Cargnegie-Mellon University.
19 L. E. Chirlian and M. M. Francl, *J. Comput. Chem.*, 1987, **8**, 894.
20 J. S. Binkley, J. A. Pople, and W. J. Hehre, *J. Am. Chem. Soc.*, 1980, **102**, 939.
21 W. F. van Gunsteren and H. J. C. Berendsen, *Molec. Phys.*, 1977, **34**, 1311.
22 I. M. Korenman and Z. G. Chernorukova, *J. Appl. Chem. USSR*, 1974, **47**, 2595, (English Translation).
23 S. Cabani, P. Gianni, V. Mollica, and L. Lepori, *J. Solution Chem.*, 1981, **10**, 563.
24 J. A. V. Butler, C. N. Ramchandani, and D. W. Thomson, *J. Chem. Soc.*, 1935, 280.
25 P. A. Bash, U. C. Singh, R. Langridge, and P. A. Kollman, *Science*, 1987, **236**, 564.