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Application of Regular Solution Theory to Biomembranes

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The solubility of small molecules in biological membranes is of importance in a number of areas including physiology, pharmacology, toxicology, and hyperbaric physiology. In spite of this widespread interest little direct attention has been paid to the problem. In this paper we present a semiempirical method based on the concepts of regular solution theory for the analysis of the solubility data of the simplest of solutes, namely inert gases. The treatment is tested against available data and is shown to provide a predictive framework for the gas solubility in a particular membrane. A more critical assessment of the treatment awaits accurate experimental work.

Recent studies of biological membranes have established the lipid bilayer as the basic structural component.[1] This bilayer has an interior hydrocarbon core some 25–35 Å thick and extensive in two dimensions.[2] Whereas the hydrocarbon chains of the phospholipids are (on time average) oriented across the plane of the bilayer, numerous studies attest to the fact that they are in an essentially fluid state, the freedom of motion increasing progressively toward the membrane interior.[3] It seems probable that for the solvation of small nonpolar molecules the lipid bilayer should be amenable to treatment as a three-dimensional liquid within certain limitations, and there are, in fact, good indications that such an approach might be successful for nonpolar solutes. For example, the strong correlation between oil solubility and the general anesthetic potency of gases[4] has long been held to imply a membranous site of action for these agents. Similarly, the permeability of membranes to nonionic solutes has been related to olive oil solubility.[5] Diamond and Wright[6] have discussed possible limitations of such a simplified approach.

Theoretical Background. Regular solution theory offers a framework for predicting solubility from a knowledge of certain parameters of the pure components.[7] Rigorous application of the theory to solutions of gases in nonpolar solvents is generally not possible, but used in a semiempirical mode, the theory has been successfully and extensively applied.

Regular solution theory characterizes solvents in terms of a solubility parameter, δ_1, defined as

$$\delta_1 = (\Delta E/V)^{1/2} \quad (1)$$

where ΔE is the molar heat of vaporization at constant volume and V is the molar volume. For dilute solutions plots of solubility *vs.* δ_1^2 for a gas in a number of solvents generally yield a regular relationship[7] (*e.g.*, Figure 1). The solubility of a gas in an additional solvent of known δ_1 can thus be predicted if solubility data for that gas in a number of other solvents are available. For solvents of limited stability and high boiling point, δ_1 cannot be evaluated from eq 1. In this case, δ_1 may be derived from solubility data using the reverse of the procedure outlined above and in Figure 1. Such solvents are often of high molecular weight and adopting this semiempirical approach is likely to be more successful in these cases because it is based directly on solubility properties and thus takes into account such factors as the large size difference between solute and solvent. The δ_1 so derived will be referred to herein as an "empirical solubility parameter" to distinguish it from the thermodynamic solubility parameter derived from the heat of vaporization of the pure solvent.

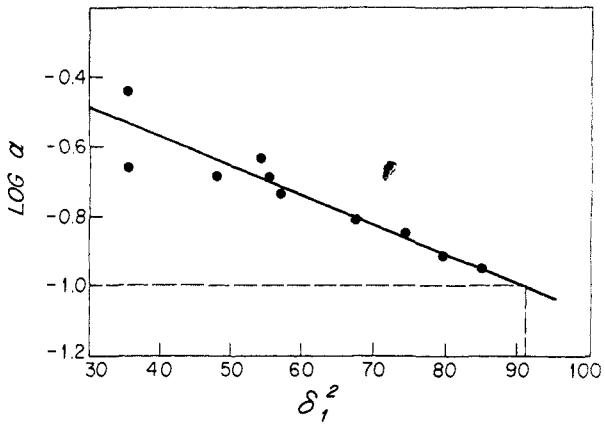

Figure 1. The relation between the Bunsen solubility coefficients (α) for nitrogen in a series of solvents and solvents' solubility parameters (δ_1 defined in eq 1). Data from ref 7. The dashed line demonstrates how the experimental solubility of nitrogen in erythrocyte ghosts can be used to define the solubility parameter.

A number of factors have to be taken into account when applying this approach to biological membranes. It is usual practice to express solubility as mole fraction, but this is not very meaningful for heterogeneous biological membranes. Instead, we have used the Bunsen coefficient (α), which is defined as the number of milliliters of gas (at STP) which dissolves in 1 ml of solvent or membrane at a partial pressure of 1 atm. Another factor is the failure of the geometric mean rule for forces between unlike molecules which causes deviations from the smooth curves usually obtained (*e.g.*, Figure 1). This is particularly true for perfluorinated compounds such as CF_4 and SF_6.[8] However, if only one class of solvent is chosen (for our purposes hydrocarbon solvents) the geometric mean rule breaks down in a systematic fashion so that smooth plots are still obtained and may be used predictively for similar solvents. The effects of size in solvents such as i-C_8H_{18} have been discussed by Hildebrand.[7] Selection of suitable solvents on this basis, however, enables reference plots of log α against δ_1^2 to be constructed for the gases of interest using data summarized in ref 7 (p 201) and 9.

Application of Regular Solution Theory to Biomembranes. In order to test whether regular solution theory may be applied to biomembranes, data for a series of gases in a membrane may be used with each of the appropriate reference plots to yield an estimate of the empirical solubility parameter as shown in Figure 1. If the theory is applicable the estimates of δ_1 given by each gas will be self-consistent.

The only suitable set of data available in the literature is that for six gases in the erythrocyte ghost membrane.[10] The experimental uncertainty in the Bunsen coefficients in that work is ±30% corresponding to an uncertainty in the estimated empirical solubility parameter of ±0.6

Table I. Results of Analysis for Erythrocyte Ghost Membrane[a]

Gas	Bunsen coeff in erythrocyte ghost[b]	Empirical solubility parameter, δ_1, for whole membrane	Empirical solubility parameter, δ_1, for membrane lipid alone
Neon	0.012	10.5	9.4
Hydrogen	0.024	10.6	9.6
Carbon monoxide	0.094	10.3	8.1
Nitrogen	0.096	9.5	7.3
Oxygen	0.113	10.1	8.3
Sulfur hexafluoride	0.151	10.5	9.9
	Mean ± S.D.	10.3 ± 0.40	8.7 ± 1.03

[a] The solubility data for CO_2 did not yield a value of δ_1 because solubility shows little dependence on the solvent solubility parameter. [b] See ref 10.

$cal^{1/2}$ $cm^{2/3}$. The empirical solubility parameters which we estimate from these data and our reference plots are given in Table I. They all fall within a range comparable to the experimental uncertainties with a mean δ_1 of 10.6 $cal^{1/2}$ $cm^{2/3}$. We conclude that regular solution theory may be applied in a self-consistent fashion to this membrane, and we may, therefore, predict Bunsen coefficients for ten additional gases for which we have reference plots using δ_1 = 10.6. These are He 0.01, Ar 0.11, Kr 0.32, Xe 1.5, CH_4 0.24, C_2H_6 1.7, C_2H_4 1.5, c-C_3H_6 5.3, CF_4 0.09, and N_2O 1.6.

Can this approach be generalized to other membranes? Measurements of the partition coefficients of benzyl alcohols[11] and spin labels[12] in lipid bilayer membranes of known compositions indicate that solvent power is a function of lipid composition and suggest that the empirical solubility parameter may vary systematically in some way with membrane composition. Characterization of δ_1 for a number of lipid bilayers might, therefore, allow the method to be applied *a priori* to any membrane of known composition, provided the heterogeneity of biomembranes can be successfully handled. That there is at least negligible specific binding to membrane protein in the red blood cell ghost by these gases is suggested by the consistency of our δ_1 estimates. This does not rule out nonspecific solvation in loosely packed hydrophobic membrane proteins. If we assume as a limiting condition membrane protein absorbs negligible solute, a lower limit to the solubility parameter of the lipid region may be fixed. In the present case, the erythrocyte ghost membrane is approximately 43 wt % lipid with roughly equal molar proportions of phospholipid and cholesterol.[13] Assuming that all the absorbed gas is concentrated in this lipid region allows one to place a mean lower limit on the empirical solubility parameter of the lipid region as 8.7 (Table I, last column). (The standard deviation of the estimate is slightly greater in this case reflecting the reduced slope of the log α *vs.* δ_1^2 plot often observed at lower δ_1.) Whether the value of 8.7 may be applied to the lipid portion of other membranes of similar compositions remains to be seen. If, however, regular solution theory can be applied to other membranes, then a wide range of applications will be available.[7] One might expect, however, that these would be limited to solute molecules small compared to the membranes dimensions.

Although not strictly comparable it is interesting to note some values of thermodynamic solubility parameters for simple solvents. Our minimum value of 8.7 for the lipid region compares to 7.3 for hexane and 8.0 for hexadecane. Hildebrand[7] has commented that the aliphatic hydrocarbons often behave as if their "practical" (*i.e.*, empirical in our terminology) solubility parameters are higher than those from heats of vaporization. The value of 10.3 for the whole membrane compares with 10.0 for carbon disulfide or 10.5 for bromoform and is higher than any values given for aliphatic hydrocarbons, suggesting that the membrane's structure may increase its cohesive energy density.

Finally, we note that the successful application of regular solution theory to a biological membrane is consistent with current notions of lipid bilayer membrane fluidity.

Conclusion

A semiempirical method, based on regular solution theory, has been proposed for predicting the solubility of gaseous solutes in membranes. Literature values for the solubility of six gases in the erythrocyte ghost membrane provide a test of the method and yield a self-consistent value of the membrane's solubility parameter. The analysis may be used to predict the solubility of other gases in this membrane.

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Annelated Thyroxine Analogs

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The diverse activity of the thyroxine molecule (1) has encouraged much synthetic effort over the last few decades (for a recent review, see ref 1). Besides the various molecular modifications that have been carried out in order to try to improve or retain thyroxine-like activity, attempts have also been made to dissociate the various activities. The work of Blank and coworkers is noteworthy in that they achieved some separation of hypocholesterolemic, cardiac, and metabolic effects.[2]