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On the use of regular solution theory as a theoretical frame for the analysis of membrane phenomena

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The structural heterogeneity of the phospholipid membrane causes difficulties in the analysis of membrane functions. These difficulties can be overcome by using probes which dissolve preferentially into one of the regions, provided that a general theory correlating probe behaviour to membrane structure exists. The use of regular solution theory (RST) as a theoretical frame for membrane analysis is examined below, and it is concluded that some, but not all, of the relations furnished by the theory are useful for membrane analysis. Using these relations, it is concluded that the hydrophobic region of the membrane has a stabilizing effect. The polar region is less stable and changes faster than the hydrophobic region. Diffusion rates are smaller in the hydrophobic region.

Introduction

The regular solution theory (RST) deals with solutions which are characterized by non-zero enthalpy and zero entropy changes. It is used extensively for the description of the solubility of non-electrolytes, and for predicting various physical parameters of solvents. These include the surface tension, the energy of vaporization, and the ratio of the thermal expansion coefficient to the isothermal compressibility coefficient of the solvent [1–3]. The basic parameters of the theory are the solubility parameters, which measure the ability of the mixing entities to go into solution. A solubility parameter of an entity is defined as the square root of its cohesive energy density.

Because of its simplicity and the many quantitative correlations which it furnishes, RST has been applied also to biological systems. The applicability of RST to the analysis of the mechanism of general anaesthetics has been suggested by Mullins [4], leading to a calculation of such derived quantities as the compressibility of the membrane from the theory [5]. In membranes, the use of RST has evolved in the wake of the intensive research carried out to clarify the mechanism of transport of various metabolytes and solutes. The theory has a potential use for the evaluation of the nature and strength of the interactions between membrane components [6–12]. However, contrary to the above expectations, there are cases where the RST does not explain the biological phenomena [13,14]. It is thus tempting to define the conditions under which this and other theories of solution can be applied to biology, and to look for places where different theoretical approaches complement each other.

In this article we discuss the applicability of the

Abbreviations: RST, regular solution theory; DMPC, dimyristoylphosphatidylcholine.

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RST to phospholipid membranes. These are well-defined systems resembling essential components of the plasma membrane in their structure and composition, and are suited for the evaluation of the affecting factors in terms of governing rules [15,17]. We show that some, but not all of the relations which appear in RST are useful. The quantities which can be deduced from the use of the framework of the RST and solubility measurements in the hydrophobic region of the membrane are those related to the cohesive energy density. Quantities which depend substantially on the entropy cannot be deduced. Strong limitations are imposed on the evaluation of membrane properties by comparison of its solubility parameters with those of model solvents.

Theoretical considerations

Solubility of gases in liquids can be regarded as consisting of two independent steps [16,18,19]:
(1) the formation of a cavity inside the liquid medium, and
(2) the accomodation of a gas molecule into the cavity.
This leads to the following equations for the standard enthalpies, entropies and free energies of solution:

$$\Delta H^0 = N(e_{ll} - e_{sl}) - RT \qquad (1)$$

$$\Delta S^0 = N(s_{ll} - s_{sl}) - R[1 + \ln(RT/V_1)] \qquad (2)$$

$$\Delta G^0 = N(f_{ll} - f_{sl}) + RT\ \ln(RT/V_1) \qquad (3)$$

For a dilute solution of rigid solute molecules, the parameters e_{ll}, s_{ll} and f_{ll} characterize hole formation and depend only on the nature of the solvent. The remaining parameters describe adsorption of the solute into the cavities and depend on the interaction between solvent and solute. The parameters obey the general relations:

$$f_{ll} = e_{ll} - Ts_{ll}$$

$$f_{sl} = e_{sl} - Ts_{sl}$$

The local energy density is given by e_{ll}/v_n, where v_h, the volume of the hole, is given by:

$$s_{sl} = 3k\ \ln[1 - (v_s/v_h)^{1/3}]$$

v_s, the volume of the solute molecule, and v_h, the volume of the hole, are two geometrical factors.

Eqns. 1–3 are general expressions deduced by thermodynamical consideration. Additional assumptions are made in the derivation of the relation between entropy and cavity size (Eqn. 4) [16]. These assumptions need not concern us, since Eqn. 4 appears here only as an experimentally justified, convenient way for deriving the hole size. The relation:

$$e_{ll}/v_h = U_1/V_1 \qquad (5)$$

yields directly the cohesive energy density of the medium in terms of the molar energy of evaporation, U_1, and the molar volume of the solvent, V_1. Eqn. 5 implies that the local energy density is equal to the average energy density. This has been examined and verified for solutions in bulk solvents [1,29] and in a phospholipid membrane [16].

The regular solution theory deals with the case where $\Delta S^0 = 0$. This occurs when all the parameters in Eqn. 2 vanish, and also when they cancel each other to give a zero total entropy. The major parameters of the RST are U_1/V_1 and the solubility parameter of the solvent, $(U_1/V_2)^{1/2}$.

Results

Our analysis on the applicability of RST to membranes is based mainly on solubility data of noble gases in the dimyristoylphosphatidylcholine (DMPC) membrane. Although the solubilities of other entities in this membrane have been recorded [12], we prefer noble gases as being easier to analyze and to treat, due to the simplicity of the solutes and to their preferential solution in the hydrophobic region of the membrane [5].

It can readily be seen from the data that the description of the system by the RST is only an approximation. The results show that the entropic contribution to the solubility is significant in comparison to the enthalpies. The ratio of entropies to enthalpies (Table I) for either cohesive or adhesive thermodynamic functions is far from being negligible. The ratio has a lower value when differences between corresponding cohesive and adhesive are considered. Most important is the ratio between

TABLE I

THE RELATIVE IMPORTANCE OF ENERGIES AND ENTROPIES IN MEMBRANE SOLUTION PROCESSES

Four processes of solute adsorption and one of hole formation are given for the DMPC membrane. Energies are given in kJ/mol and entropies in J/mol per K. Δs and Δe are differences in values between the adhesive parameters and the value characterizing hole formation. The parameters are derived from solubility data of noble gases. The method of measurement of these solubilities is given in Ref. 12. The solubility and the standard free energy of solution are related by the equation. $\bar{\mu}^0 = RT \ln(p/X_s)$, where p and X_s are, respectively, the partial pressure of the solute in the gas phase above the solution and its mole fraction inside the solution. The standard enthalpies and entropies were derived from the temperature dependence of the standard free energy [12]. The e and s parameters are derived from Eqns. 1–3 [16].

Process	s	e	Δs	Δe	Ts/e	$\lvert T\Delta s/\Delta e \rvert$
Ne	31.2	7.18	23.8	24.03	1.30	0.3
Ar	58.9	13.42	−3.9	17.79	1.32	0.06
Kr	69.2	15.80	−14.2	15.41	1.31	0.28
Xe	88.9	18.73	−33.9	12.48	1.42	0.81
Cohesive	55.0	31.21	–	–	0.53	–

the cohesive parameters, since it is three parameters which reflect the nature of the solvent system and correlate with the physical properties of the solvent. The ratio Ts_{ll}/e_{ll}, given in Table II for various bulk solvents and for the hydrophobic region of the membrane, shows that the use of RST as a general theory describing the behaviour of membranes is not justified. The RST is valid only when used to extract physical quantities that are independent of entropy. Most noticeable among the parameters that either cannot be used, or that have lost their theoretical meaning is the solubility parameter. Its use as the sole parameter affecting solution is based on the assumption that only energetic interactions affect the behaviour of the solution, but as we have just shown, this assumption is not supported by the experimental findings. Another important quantity whose deduction from the RST is not justified for the membrane is surface tension (Katz, Y., Ref. 36, in press). A correlation between surface tension and the solubility parameter has been established which is applicable to many systems. This correlation cannot, however, be applied to membranes, where the relative importance of the entropy is greater. We will show below that while the RST is not general, it is still possible to create an apparent solubility parameter which fits the solubility data quite well (Fig. 1). This apparent solubility parameter cannot be used to obtain the cohesive energy

TABLE II

PARAMETERS CHARACTERIZING HOLE FORMATION IN VARIOUS ISOTROPIC SOLVENTS AND IN THE HYDROPHOBIC REGION OF THE DMPC MEMBRANE

Entropies are given in $J \cdot K^{-1} \cdot mol^{-1}$, energies in kJ/mol, and molar hole volumes in cm^3/mol. Literature values $(e_{ll}/v_h)_{lit}$ are from Hildebrand and Scott [1]. The parameters s_{ll} and e_{ll} were derived from the standard entropies and enthalpies of solution, using the procedures described in Refs. 16–8. It can be seen that for most solvents the difference between calculated and literature values is less or about 10%, for benzene it is about 30%.

Solvent	Ns_{ll}	Ne_{ll}	Nv_h	e_{ll}/v_h	$(e_{ll}/v_h)_{lit}$	Ts_{ll}/e_{ll}
DMPC	55.00	31.21	25.43	1.23	–	0.53
n-Hexane	15.46	16.59	65.64	0.25	0.22	0.28
n-Dodecane	11.17	16.90	65.56	0.26	0.25	0.20
Cyclohexane	24.03	18.64	62.35	0.30	0.28	0.32
Benzene	27.38	23.06	50.90	0.45	0.35	0.36

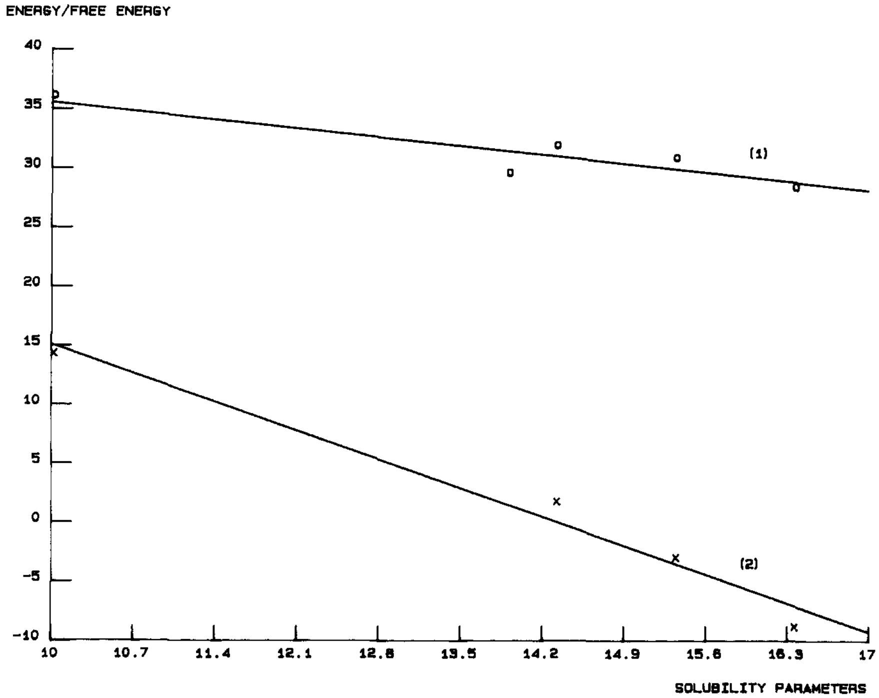

Fig. 1. The difference between true and apparent cohesive energy densities. The slope and intercept have a meaning of cohesive energy density and solubility parameter of the solvent, respectively. It is seen that for the hydrophobic region of the membrane apparent and true cohesive energy densities differ appreciably. The solvent used is the hydrophobic region of the DMPC membrane and the solutes are: Ne, Ar, Kr, Xe and CH_4. The data for the enthalpy (curve 2) and for the free energy (curve 1) are from Refs. 17, 12 and 10. Solubility parameters are from Hildebrand and Scott [2]. Enthalpies and free energies are in kJ/mol and solubility parameters are in $(J/cm^3)^{1/2}$.

density and the physical factors related to it. A discrepancy between theory and experiment was observed also by Simon et al. [13], when comparing the partition coefficient of *n*-hexane into phospholipid bilayers with that for various bulk solvents, and employing the RST to explain the peculiarities of membrane solubility. However, these authors worked with a relatively complex solute having several internal degrees of freedom. It is therefore difficult to use their results for further analysis. The use of simpler solutes, such as the spherically symmetric noble gases, provide a better means for testing the RST. The cohesive energy density (and hence the real solubility parameter) can be calculated once the entropy is taken into account.

Our calculations, based on the simultaneous use of Eqns. 1–5 and experimental determination of noble gas solubility, show that the cohesive energy density is 1.23 kJ/cm^3. Handbook data were used for v_s. The experimental results are used to derive s_{ll}, e_{ll} and v_h. Hole volumes based on entropy measurements of Ne, Ar, Kr and Xe in the membrane gave estimated molar hole volumes of 25.44 ± 1.39 cm^3/mol. Results which differ somewhat from these are derived when other methods of estimation are used [16,17]. Estimation of the molar hole volume from enthalpy measurements gives a value of 20.43 cm^3/mol. Molar hole volumes in bulk solvents were also deduced from molar heats of vaporization, yielding values that are higher by about 12% than those which are derived using Eqn. 4. Comparing the results derived by the various methods [16,17], we observe that the value derived from entropy measurements is correct within 20%. This is also the accuracy which has to be assumed when the values of the cohesive energy density are used. As can be seen from Table II, the cohesive energy density of the DMPC membrane is very high in comparison to the values characterizing the hydrophobic bulk solvents [20,21,36]. This unusually high value reflects greater interaction per unit volume of the membrane.

Using Eqn. 5 and 228 cm^3 as the molar volume of one acyl chain residue of the DMPC molecule (we consider an acyl residue of 12 methylene and one methyl groups at a density of 0.80 9/cm^3), we find that the molar heat of vaporization of a single and a double hydrocarbon chain of the hydrophobic residue of dimyristoylphosphatidylcholine are 278.8 or 557.6 kJ/mol, respectively. A higher value is obtained when the contributions of the internal degrees of freedom to the enthalpy are taken into account. For the DMPC membrane, the number of methylene groups in the trans state was estimated to be 7–19 per DMPC molecule [22]. When fixed in the trans state, the energy of a methylene group is lower by 2 kJ/mol than the value which it has in the free state. Thus, a contribution of 14.7 to 39.9 J/mol is expected from the conformation. As can be seen, this contribution is less than 8% of the total energy. By contrast, the energy of vaporization of *n*-tetradecane, which has the same composition and a compartable molecular weight, is only 69.9 kJ/mol. The difference reflects the great cohesivity of the membrane and its ability to withstand disruption (Some investigators claim that the first methylene group of the acyl residue of the DMPC molecule has a diminished interaction. This will lower the estimated energy of vaporization by about 8%, even then the cohesivity of the membrane is significantly greater than the cohesivity of the bulk solvents [23].) The stabilizing enthalpic effect is counteracted by the entropy of the membrane which, as shown above, makes a relatively large contribution to the free energy. Nevertheless, the energy is still large enough to play the dominant role.

The relation between the cohesive energy density of the membrane and the important ratio of expansivity to compressibility is obtained directly by applying thermodynamics to the definitions [24]:

$$\kappa = -(1/V)(\partial V/\partial p)_T \tag{6}$$

$$\alpha = (1/V)(\partial V/\partial T)_p \tag{7}$$

TABLE III

COMPRESSIBILITIES AND THERMAL EXPANSION COEFFICIENTS FOR VARIOUS SOLVENTS

Reference is made to the measured values of the total DMPC membrane [27]. Hydrophobic region denotes the α/κ value deduced for the hydrophobic region of the DMPC membrane. Compressibilities and expansion coefficients for the bulk solvents were extracted from the Handbook of Physics and Chemistry [25]. The values for the hydrophobic region were calculated from the cohesive energy density using Eqn. 8. An error of less than 20% is expected in the calculated values, most of it made in the calculation of the hole volume and a much lower contribution from the evaluated cohesive parameter e_{ll} [16]. Direct measurements of expansivity done in a vibrating quartz psychometer and of the adiabatic compressibility by sound velocity measurements made on phospholipid bilayers and on the purple membrane of the *Halobacterium halobium* were accurate to within 15% [27].

Solvent	κ (atm^{-1}) ($\times 10^5$)	κ/κ_D	α (K^{-1}) ($\times 10^3$)	α/α_D	(α/κ) (atm/K) ($\times 10^4$)	$(\alpha/\kappa)/(\alpha/\kappa)_D$
DMPC	4.34	1.0	0.88	1.0	20.28	1.0
Purple membrane	2.30	0.5	0.63	0.7	26.30	1.3
CCP$_4$	10.46	2.4	1.20	1.4	11.46	0.6
Isopropanol	10.20	2.4	1.03	1.2	10.12	0.5
Ethyl acetate	9.13	2.1	1.30	1.5	14.23	0.7
Water	4.61	1.1	0.15	0.2	3.25	0.2
n-Hexane	16.00	3.7	1.29	1.5	8.05	0.4
n-Dodecane	9.00	2.1	0.95	1.1	10.53	0.5
n-Tetradecane	8.20	1.9	0.90	1.0	11.02	0.5
n-Hexadecane	7.40	1.7	0.89	1.0	12.03	0.6
Cyclohexane	12.30	2.8	1.14	1.3	9.27	0.5
Benzene	9.40	2.2	1.21	1.4	12.87	0.6
Hydrophobic region	–	–	–	–	41.00	2.0

Rearrangement and the use of thermodynamics give:

$$(\partial U/\partial V)_T = T(\partial p/\partial T)_V - p = T(\alpha/\kappa) \tag{8}$$

The ratio of the thermal expansion coefficients α, to the isothermal compressibility, κ, which is obtained for the hydrophobic region of the membrane, using Eqn. 8, is significantly greater than the values for the bulk hydrophobic solvents. It can be seen from Table III that the ratio for these membranes is about 4-times higher than the values for the bulk solvents [25].

Two systems where direct measurements of compressibility and thermal expansion have been made are the DMPC bilayer and the purple membrane of the *Halobacterium halobium* [26,27]. The ratio of thermal expansion to isothermal compressibility derived for these membranes by direct measurement is only about 2-times greater than that which characterizes the bulk solvents, or about half the value obtained from noble gas measurements (Table III). To our opinion these differences reflect the fact that direct compressibility and expansion measurements relate to the whole membrane, whereas values deduced from the solubility of the noble gases relate exclusively to the hydrophobic region. The compressibility and expansion for the whole membrane are given by:

$$\alpha = [1/(V_1 + V_2)][(\partial V_1/\partial T) + (\partial V_2/\partial T)]_p \tag{9a}$$

and

$$\kappa = [1/(V_1 + V_2)][(\partial V_1/\partial p) + (\partial V_2/\partial p)]_T \tag{9b}$$

where the subscripts 1 and 2 denote the hydrophobic and hydrophilic regions, respectively. This gives:

$$\alpha/\kappa = (\alpha_1 V_1 + \alpha_2 V_2)/(\alpha_1 V_1 + \alpha_2 V_2) \tag{10}$$

which is converted, by simple algebraic manipulation, into the relation:

$$\alpha/\kappa - \alpha_1/\kappa_1 = (\kappa_2 V_2/\kappa_1 V_1)[(\alpha_2/\kappa_2) - (\alpha/\kappa)] \tag{11}$$

The experimental finding that α/κ for the hydrophobic region is greater than the value found for the membrane as a whole, and the fact that $\kappa_2 V_2/\kappa_2 V_1$ is definitely positive, imply that: $\alpha_2/\kappa_2 < \alpha/\kappa < \alpha_2/\kappa_1$. Inserting this result into Eqn. 8, we see that the cohesive energy density in the hydrophobic region is greater than in the hydrophilic region. Since $3V_2 > V_1 > 2V_2$, it follows that the regional cohesive energy and regional cohesive energy density are also greater in the hydrophobic region, which is in agreement with the claims made above.

The increased value which we have found for the ratio between the compressibility and thermal expansion in the hydrophobic region can be attributed to either an elevated value of the thermal expansion, or to a diminished value of the compressibility coefficient. We do not have sufficient experimental data to conclude which of these possibilities occurs, but we can use theoretical considerations such as those employed by Furth [28], or in the scaled particle theory [29]. These equations, which were developed for simple particles, are naturally approximate and of limited applicability; nevertheless, they are qualitatively correct and therefore sufficient to show the general trend. According to Furth, the fluctuation in volume is directly proportional to the volume of the hole that the thermal motion of the molecules create in the medium. The fluctuation in entropy is a function of the hole volume. Since the expansion and compressibility coefficients are always related to fluctuations in the properties of the system by thermodynamic relations given in [30], it follows that:

$$\kappa = \overline{\Delta V^2}/V_1 kT \tag{12}$$

$$\alpha = \Delta\overline{S\Delta V}/V_1 kT \tag{13}$$

which together give the ratio α/κ as:

$$\alpha/\kappa = \overline{\Delta S\Delta V}/\overline{\Delta V^2} \tag{14}$$

We obtain that:

$$\alpha_1/\kappa_1 = s_{\mathrm{ll}} v_{\mathrm{h}}/v_{\mathrm{h}}^2 = s_{\mathrm{ll}}/v_{\mathrm{h}} \tag{15}$$

The calculated results in Table IV agree qualitatively with the experimental results showing that the theoretical predictions of Furth are essentially correct for liquids to be used for analysis and comparison. It therefore follows from the results of Table IV that it is the diminished compressibil-

TABLE IV

RATIOS OF EXPANSION TO COMPRESSIBILITY COEFFICIENTS CALCULATED FROM FLUCTUATIONS

Volumes are given here in cm^3, entropies in J$\cdot$mol$^{-1}\cdot$K^{-1}. D denotes the hydrophobic region of the DMPC membrane.

Solvent	$s_{\mathrm{II}}\cdot v_{\mathrm{h}}$ ($\times10^{-3}$)	$\left\{\frac{s_{\mathrm{II}}v_{\mathrm{hD}}}{s_{\mathrm{II}}v_{\mathrm{h}}}\right\}$	v_{h}^2 ($\times10^{-3}$)	$\left\{\frac{v_{\mathrm{hD}}^2}{v_{\mathrm{h}}^2}\right\}$	$\left\{\frac{s_{\mathrm{II}}}{v_{\mathrm{h}}}\right\}$	$(\alpha/\kappa)_{\mathrm{exp}}$	$\left\{\frac{(s_{\mathrm{II}}/v_{\mathrm{h}})_{\mathrm{D}}}{(s_{\mathrm{II}}/v_{\mathrm{h}})}\right\}_{\mathrm{exp}}$	
n-Hexane	1.01	1.39	4.31	0.15	0.24	0.8	0.91	10.00
n-Dodecane	0.73	1.92	4.30	0.15	0.17	1.05	12.7	7.8
Cyclohexane	1.50	0.93	3.89	0.17	0.39	0.91	5.6	8.8
Benzene	1.39	1.00	2.59	–	0.54	1.28	4.0	6.3
DMPC	1.4	–	0.65	–	2.16	–	1.0	–

ity of the hydrophobic region which is unique. The expansion coefficient is about the same as in other solvents. The diminished compressibility means that fluctuations in the density of the hydrophobic region are relatively small. Calculating hole volumes from the magnitude of the parameters characterizing the solubility of the noble gases in various hydrophobic solvents, we notice that the smallest values are in the hydrophobic region of the phospholipid membrane. The relatively small change in volume which characterizes hole formation in the bilayer is accompanied by an entropy change that is several times greater than corresponding entropy changes inside the bulk hydrophobic solvents.

Certain additional factors, such as conformational changes, also exist in the bilayer, and may contribute to the magnitude of the ratio compressibility to thermal expansion in the hydrophobic region. As was shown above, such an effect will increase even further the ratio of expansion to compressibility in the hydrophobic region.

Discussion

From what is written above, it follows that the hydrophobic region of the membrane does not have the characteristics of a regular solution, and the doubts about the use of the RST for this region are justified to a certain extent, but it also follows that we can still use relations that were derived by this theory, provides that we are aware of their scope and limitations. Below, we consider these points in detail and show in turn that:

(1) The errors introduced by the conventional methods of analysis are due to two causes, neglection of entropic contributions and the assumption that the internal degrees of freedom do not affect the outcome of the experimental values.

(2) Parameters of the RST can be used either for correlating between solubilities or for deriving physical properties, but not for both purposes simultaneously. In order to achieve both aspects correctly, a distinction has to be made between true and apparent cohesive energy densities.

(3) It is the true cohesive energy density which is related to the physical properties of the membrane by the relations used above. It can be furnished only from the temperature dependence of the solubilities.

(4) The properties derived from the cohesive energy density of the membrane are important to the understanding of membrane function and at present can be deduced only from solubility data.

The use of RST to deduce physical properties of the membrane is least suitable when these properties are strongly related to the entropy of the system. For example, there is a strong correlation between the cohesive energy density of a solvent and its surface tension [1], but the existing analogies cannot be used to deduce the surface tension of the membrane which is affected by the large entropy of the membrane [20]. We have seen above that a similar argument holds also for solubility measurements. Solubility cannot be deduced from the known solubility parameters of the solute and membrane because the solubility depends strongly on the entropy, whereas the solubility parameters $(U/V)^{1/2}$ reflect only the enthalpy. Another limitation of the RST in its present form is that it takes into account only the intermolecular interactions. Thus, the cohesive energy density which is obtained from noble gas solubility in the hydrophobic region of the membrane may deviate from the one deduced from the ratio of thermal expansion to isothermal compressibility. In the later, contributions due to changes in the internal degrees of freedom (e.g., change in the freedom for internal rotations) may play a role, whereas noble gas solubility depends only on the intermolecular dispersive interactions between chains [17]. Similar reasons may cause membrane cohesive energy densities obtained from paraffin solubility to differ from the values deduced when the solutes are the noble gases [15]. Because the cohesive energy density depends on the degrees of freedom which affect the given measurement, attention must be paid to the nature of the experiment, even when the relations of the RST hold. The extension of the RST to overcome this problem is straightforward, however, since each contribution to the energy can be separately treated. Furthermore, a possibility for a more penetrating analysis of membrane behaviour is offered by this extension. For example, the comparison of the paraffin solubility with noble gas solubility in order to furnish the net effect of internal rotations on membrane behavior.

The findings imply that an apparent cohesive energy density of the hydrophobic region can be derived from the solubility data by plotting free energies of solution versus solubility parameters of the solutes. Because the entropies and enthalpies of solution are linearly related [16], a straight line is established where the intercept and the slope of the line have the dimensions of cohesive energy density and of solubility parameter, respectively. These constants have no direct physical meaning and therefore are not suitable for deriving the physical properties of the membrane. The constants are nevertheless useful, since they can be used to correlate between solubilities and to furnish unknown solubilities from the correlation. A true cohesive energy density can be derived only from the temperature dependence of the solubility (i.e., from the enthalpies and entropies of solution), and it can be used to derive physical properties of the hydrophobic region. It is useless for predicting membrane solubilities. The difference between the two kinds of cohesive energy densities is demonstrated in Fig. 1. Two conclusions follow. First that it is not the use of the RST for membrane analysis which is wrong, but rather its improper employment. Second that the indiscriminate use of true and apparent cohesive energy densities, which is commonly made in the analysis of some biological phenomena [4,10], is not justified.

Two useful values have been derived from the true cohesive energy density using relations employed in the RST. These are the energy of vaporization and the ratio between the thermal expansion coefficient and the isothermal compressibility coefficient. Together they give the strength of interactions inside the hydrophobic region, its stability, and its contribution to membrane dynamics, and are therefore useful for understanding such phenomena as phase transition, binding of lipid to membranes, and transbilayer diffusion. The method used here to derive these quantities has the advantage that selective solubility allows to probe independently the different regions of the membrane. For the physical properties depicted here to be critical in determining membrane function, three conditions must be fulfilled: (1) a large variability in the composition of the hydrophobic moieties of biological membranes; (2) a sensitivity of membrane function to membrane composition; and (3) a strong dependence of the physical properties considered on membrane composition. The existence of the first two conditions is demonstrated repeatedly in the literature [34] [35]. Here we show the strong influence of membrane composition on the physical properties of the membrane. This is done by comparing two extremes, bulk solvents and the gel state of the membrane. In the first the molecules are disordered and in the second they are highly ordered. A comparison shows that the measured values vary over a wide range, being much larger in DMPC than in the bulk solvents which are used here. An estimate exists which shows that for the gel state the cohesive energy density is greater than that of the liquid crystal by about 10% [17]. The cohesive energy density is about 4–5-times greater in the membrane, and the cohesive energy per hydrocarbon chain is about 4–times greater than the value found in comparable bulk hydrophobic solvents. These reflect a great stability of the hydrophobic region of the membrane and an ability to withstand disruption. Table I shows that the stabilizing effect is counteracted by a relatively large entropy contribution which acts to lower the free energy. The ratio of energy to entropy is about two.

The compressibility of a medium is directly related to the fluctuation in density and this relation is given in Eqn. 12. It is easy to show that $\alpha_1 \approx \alpha_2$ so when $\alpha_1/\kappa_1 \approx 2\alpha/\kappa$, we get that $\kappa_2 \approx \Delta\kappa_1$ (see Appendix). Thus, we see that the fluctuation in density in the hydrophilic region is significantly greater than it is in the hydrophobic region. The estimated compressibilities are given in the phases of Table III. Interpreted in terms of non-equilibrium thermodynamics, the difference in compressibilities means that the hydropholic region undergoes change more readily and is less stable than the hydrophobic region [33]. It also implies that diffusion rates are smaller in the hydrophobic region and larger in the hydrophilic region.

A final comment concerns the common method of applying bulk solvents as models for the membrane and to deduce the properties of the hydrophobic region of the membrane from the known properties of the common bulk, non-polar solvents [31,32]. This procedure is not justified because, as is shown here, membrane order plays a substantial role in membrane processes, a role which is significantly greater than found in bulk solvents. Use of bulk solvents is therefore made in this article only for two purposes, to define the range of possible membrane value of the physical properties mentioned above and to examine the validity and meaning of relations proposed by the RST.

Appendix

Given the findings that:

$$\alpha_1 = \alpha_2 \tag{A-1}$$

$$V_1 = 2V_2 \tag{A-2}$$

$$\alpha_1/\kappa_1 = \alpha/\kappa \tag{A-3}$$

it can be shown from Eqns. A-1, A-3 and 9a that:

$$\kappa = 2\kappa_1 \tag{A-4}$$

from Eqns. 9b and A-3 we have:

$$2\kappa_1 = (\kappa_1 V_1 + \kappa_2 V_2)/(V_1 + V_2) \tag{A-5}$$

which gives when inserting Eqn. A-2:

$$2\kappa_1 = (2/3)\kappa_1 + (1/3)\kappa_2 \tag{A-6}$$

or

$$\kappa_2 = 4\kappa_1 \tag{A-7}$$

Eqn. A-7 being the result used in the discussion above.

References

1 Hildebrand, J.H. and Scott, R.L. (1964) The Solubility of Nonelectrolytes, Dover, New York.
2 Hildebrand, J.H., Prausnitz, J.M. and Scott, R.L. (1970) Regular and Related Solutions, Van Nostrand-Reinhold, New York.
3 Battino, R. and Clever, H.L. (1966) Chem. Rev. 66, 395–163.
4 Mullins, L.J. (1954) Chem. Rev. 54, 289–323.
5 Lever, M.J., Miller, K.W., Paton, W.D.M. and Smith, E.B. (1971) Nature 368, 71.
6 Diamond, J.M. and Wright, E.M. (1969) Annu. Rev. Physiol. 31, 581.
7 Diamond, J.M. and Katz, Y. (1971) J. Membr. Biol. 17, 121.
8 Trauble, H. (1971) J. Membr. Biol. 4, 193–208.
9 Simon, S.A., Stone, W.L. and Busto-Lattore, P. (1977) Biochim. Biophys. Acta 469, 378–388.
10 Miller, K.W., Hammond, L. and Porter, E.G. (1977) Chem. Phys. Lipids 20, 229.
11 Pagano, R.E. and Gershfeld, N.L. (1972) J. Colloid Interface Sci. 41, 311–318.
12 Katz, Y. (1981) Biochim. Biophys. Acta 647, 119–129.
13 Simon, S.A., Stone, W.L. and Bennett, P.B. (1979) Biochim. Biophys. Acta 550, 38–47.
14 Katz, Y. and Simon, S.A. (1977) Biochim. Biophys. Acta 471, 1–15.
15 Katz, Y., Hoffman, M.E. and Blumenthal, R. (1983) J. Theor. Biol. 105, 493–505.
16 Katz, Y. (1985) J. Chem. Soc. Faraday Trans. I, 81, 579–596.
17 Katz, Y. (1986) J. Phys. Chem. 90, 190–195.
18 Eley, D.D. (1939) Trans. Faraday Soc. 35, 128.
19 Pierotti, R.A. (1963) J. Phys. Chem. 67, 1840.
20 Lecuyer, H. and Dervichian, D.G. (1969) J. Mol. Biol. 45, 39.
21 Nagle, J.F. and Wilkinson, D.A. (1978) Biophys. J. 23, 159–175.
22 Nagle, J.F. (1973) J. Chem. Phys. 58, 252–264.
23 Bocian, D.F. and Chan, S.I. (1978) Annu. Rev. Phys. Chem. 29, 307–335.
24 Guggenheim, E.A. (195) Thermodynamics, North-Holland, Amsterdam.
25 Handbook of Chemistry and Physics (1974) 55th Edn., CRC, Boca Raton, FL.
26 Nagle, J.F. and Wilkinson, D.A. (1978) Biophys. J. 23, 159–175.

27 Marque, J., Eisenstien, L., Graton, E., Sturtevant, J.M. and Hardy, C.J. (1984) Biophys. J. 46, 567–572.
28 Furth, R. (1984) Proc. Cambridge Phil. Soc., 252–275.
29 Riess, H. (1966) Adv. Chem. Phys. 9, 1–84.
30 Landau, L.D. and Lifschitz, E. (1958) Statistical Physics, Pergamon, Oxford.
31 Stein, W.D. (1981) in Membrane Transport, Vol. 2 (Bonting, S.L. and De Pont, J.J.H.H.M., ed.), pp. 1–28, Elsevier/North-Holland, Amsterdam.
32 Finkelstein A. (1976) J. Gen. Physiol. 67, 45.
33 De Groot, S.A. and Mazur, P. (1962) Nonequilibrium Thermodynamics; North-Holland, Amsterdam.
34 Stubbs, C.D. (1983) Essays Biol. 19, 1–39.
35 Blumenthal, R. (1987) Curr. Top. Membr. Transp. 29, 203–255.
36 Katz, Y. (1988) J. Colloid Interface Sci., in press.