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Swelling of Phospholipid Bilayers by Solvents

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The equilibrium partitioning of solvent between water and phospholipid vesicles above the phase transition temperature of the vesicles is treated in a manner similar to that previously successful in describing solvent partitioning between water and polymer latex particles. The mixing of solvent and the alkane part of the phospholipid vesicles is described by classical Flory–Huggins theory. The vesicle surface free energy is described by the Gibbs–Thomson equation. The subsequent model has a single adjustable parameter, the saturation concentration of solvent in vesicles, that is restricted within physical limits. Comparisons of theory and experiment show good agreement and allow calculations of vesicle interfacial tensions.

Introduction

The swelling of vesicles and surfactant or phospholipid bilayers with solvents has been the subject of sporadic interest,[1–3] despite the fact that it is a subject that possibly holds the key to the nature of vesicles. Surfactant and phospholipid bilayers are often used as model systems in biological studies and also in surfactant and colloidal studies. These species have also been studied because of their ability to absorb and release drugs in medical applications. Recently, we have performed a free radical polymerization of unsaturated oil soluble monomer within the interior of vesicles in order to make hollow polymer (latex) particles.[4] Many of the above listed studies require a knowledge of how solvents are absorbed into vesicles and how these solvents partition between the bilayer (vesicle) and aqueous phases.

It has often been stated that the swelling of phospholipid bilayers and vesicles cannot be explained in terms of bulk thermodynamic models.[5,6] There are at least two reasons for these statements. First, bilayers have high surface to volume ratios and are therefore often thought of as interfacial phases of matter rather than bulk phases. Secondly, bilayers are considered to have nonuniform structure, which is not the case for bulk phases. For these and other reasons much effort has been expended in modeling the swelling of surfactant bilayers by solvents in a manner that ignores bulk thermodynamics. Statistical mechanical approaches to the problem have been most common.[7,8] These often have good success but may suffer from being somewhat qualitative in nature or by requiring the evaluation of unmeasured parameters. These facts limit the predictive power of these models.

In this paper analogies are drawn between the swelling of latex particles by solvents and the swelling of phospholipid vesicles by solvents. The saturation swelling of latex particles by solvents is admirably described by the Morton equation,[9,10] and the partial swelling of latex particles is described by simple adaptation of this equation.[10–12] The basis of these equations is that the mixing of solvents and polymer is readily described by Flory–Huggins theory. In this paper we extend this approach to phospholipid vesicles and develop analogues to expressions previously described for the latex particle situation. The mixing of solvent and the alkane part of a phospholipid in vesicle bilayers is thought of in bulk thermodynamic terms. Obviously this does not allow us to consider the microstructure of the swelling phenomena within vesicle bilayers. But we state from the outset that our primary goal is to develop a model that predicts only the absolute concentration of solvent within a vesicle bilayer, both at partial and at saturation swelling, and therefore, also the solvent partition coefficient.

Finally, listed below are the limitations of the systems that we are interested in. (1) We consider only swelling of vesicles by a single solvent. Trivial extensions can be made to deal with two or more solvents. These extensions are analogous to theories previously developed for the swelling of latex particles by two or more solvents.[13,14] This will be done in a following publication. (2) We consider only partially water soluble solvents that are good solvents for the alkane part of the phospholipid vesicles. (3) All work in this paper is for phospholipid vesicles, but the considerations are quite general and can be applied to all bilayer structures with simple modifications. (4) Finally, only phospholipid vesicles above the phase transition of the phospholipid chains are considered. Hence the mixing of the alkane part of the phospholipid chains and solvents results in a single phase.

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(15) (a) Piton, M. Personal communication. (b) The lateral diffusion coefficient of a C12 surfactant in a vesicle bilayer as measured by pulsed field gradient NMR is 1 order of magnitude less than the mobility of a solvent molecule in the vesicle.[15] A phospholipid molecule has much greater steric hindrance than a C12 species; therefore it is considered that the lateral diffusion coefficient of phospholipid species in bilayers will be at least 2 orders of magnitude less than that of a solvent molecule.

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Theory

In this section we first consider the swelling of latex particles by solvent and then derive analogous expressions for phospholipid vesicles.

Morton et al.[9] considered the saturation swelling of latex particles by solvents having limited solubility in the water phase. When the swollen latex particle is in equilibrium with the free solvent phase, the partial molar free energy of the solvent is given by

$$\Delta G = \Delta G_m + \Delta G_s = 0 \quad (1)$$

where ΔG is the partial molar free energy of solvent, ΔG_m the contribution from the energy of mixing of solvent and polymer, and ΔG_s the contribution from the particle–water interfacial energy.

Morton et al. expressed the free energy of mixing of solvent and polymer in terms of the classical Flory–Huggins theory:[9]

$$\Delta G_m/RT = \ln(1 - v_p) + v_p\left(1 - \frac{1}{M_n}\right) + \chi v_p^{\,2} \quad (2)$$

where v_p is the volume fraction of polymer in the latex particles, M_n the number average degree of polymerization, R the gas constant, T the temperature, and χ the Flory–Huggins interaction parameter.

The interfacial free energy was given in terms of the Gibbs–Thomson equation:

$$\Delta G_s = \frac{2V_m\gamma v_p^{\,1/3}}{R_o} \quad (3)$$

where V_m is the partial molar volume of the solvent, γ the particle–water interfacial tension, and R_o the unswollen radius of the latex particle.

Combining the terms above gives the so-called Morton equation at saturation swelling:[9–11]

$$\ln(1 - v_{p,sat}) + v_{p,sat}\left(1 - \frac{1}{M_n}\right) + \chi v_{p,sat}^{\,2} + \frac{2V_m\gamma v_{p,sat}^{\,1/3}}{R_oRT} = 0 \quad (4)$$

where $v_{p,sat}$ is the volume fraction of polymer in the latex particles at saturation swelling by solvent.

An analogue for the Morton equation that deals with partial swelling of latex particles has been developed. If the latex particles are not saturated by solvent then there is no pure solvent phase present (i.e., no solvent droplets). The partial molar free energy of the solvent in the latex particle phase is then given by[11,12]

$$\Delta G = RT\ln(a) \quad (5)$$

where a is the activity of the solvent given by[14]

$$a = \frac{\alpha[M]_{aq}}{\alpha^\circ[M]_{aq}^\circ} \quad (6)$$

Here, $[M]_{aq}$ is the concentration of solvent in the aqueous phase and $[M]_{aq}^\circ$ is the saturation concentration of solvent in the aqueous phase at an arbitrary standard state, α is the activity coefficient of the solvent, and α° is the activity coefficient of the solvent at an arbitrarily chosen standard state. If we choose the standard state to be the saturation concentration of the solvent in the aqueous phase, and note that for low concentrations of solvent (less than a few molar) that $\alpha = \alpha^\circ$, we find[14]

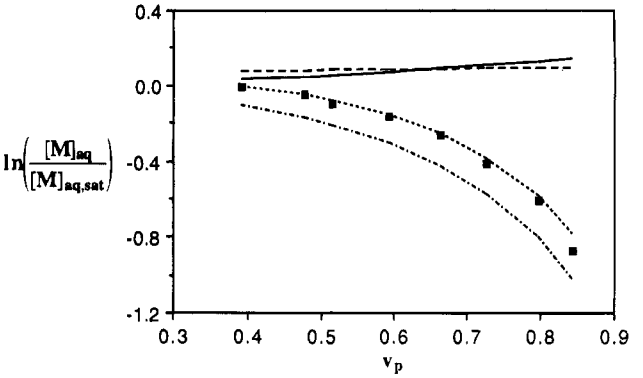

Figure 1. Comparison of theoretical predictions and experimental measurements of methyl acrylate partitioning between water and poly(styrene-*co*-methyl acrylate) latex particles at 45 °C. Theoretical predictions: configurational entropy term given by $\ln([M]_{aq}/[M]_{aq,sat}) = \ln(1 - v_p) + v_p(1 - 1/M_n)$(- · -); residual free energy term given by $\ln([M]_{aq}/[M]_{aq,sat}) = \chi v_p^{\,2}$, with $\chi = 0.2$ (—); interfacial free energy term given by $\ln([M]_{aq}/[M]_{aq,sat}) = 2V_m\gamma v_p^{\,1/3}/R_oRT$, with $\gamma = 45$ dyn·cm^{-1} (– – –); and eq 8 with $\chi = 0.2$ and $\gamma = 45$ dyn·cm^{-1} (· · ·). Data from ref 11.

$$a = \frac{[M]_{aq}}{[M]_{aq,sat}} \quad (7)$$

where the $[M]_{aq,sat}$ is the saturation concentration of the solvent in the aqueous phase.

The final result for partial swelling of latex particles by solvent is[11,12]

$$\ln(1 - v_p) + v_p\left(1 - \frac{1}{M_n}\right) + \chi v_p^{\,2} + \frac{2V_m\gamma v_p^{\,1/3}}{R_oRT} = \ln\left(\frac{[M]_{aq}}{[M]_{aq,sat}}\right) \quad (8)$$

The solvent partitioning between the aqueous and latex particle phases can be predicted from eq 8. However, this requires that both the interaction parameter and the interfacial tension be known. A further complication is that both of these parameters may be volume fraction polymer dependent. Also, values of these parameters are difficult to determine by independent experiments.

Partitioning results for solvents at *partial* swelling of latex particles show that the interfacial free energy has little effect upon the changes in partitioning of solvent. To emphasise this point, in Figure 1, the predicted contribution of all three terms in eq 8 are displayed individually for partial swelling of a latex particle by solvent. These terms are the configurational entropy of mixing of polymer and solvent term ($\Delta G_m = \ln(1 - v_p) + v_p(1 - 1/M_n)$, derived by considering the entropy of mixing of an assembly of random-flight chain molecules with solvent), the "residual" free energy term ($\Delta G_m = \chi v_p^{\,2}$, containing both enthalpic and entropic terms), and the interfacial free energy term ($\Delta G_s = 2V_m\gamma v_p^{\,1/3}/R_oRT$). It is immediately obvious from these results that at high-volume fractions of polymer the dominant contribution to equilibrium is the configurational entropy of mixing of solvent and polymer. The sum of all terms admirably reproduces experimental data (Figure 1).

Realizing the above result, a semiempirical approach was developed[11] in which the sum of the residual free energy and the particle–water interfacial free energy terms in eq 8 are considered approximately constant at

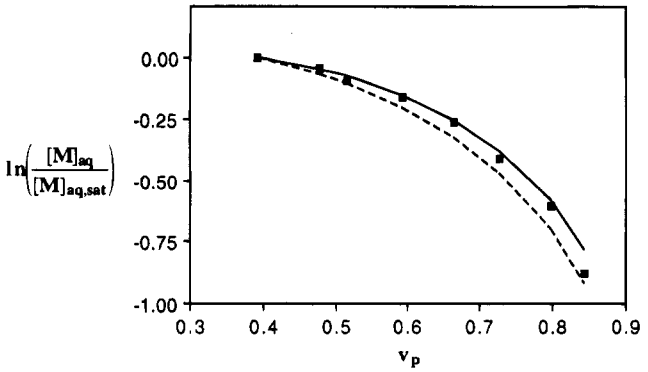

Figure 2. Comparison of theoretical predictions and experimental measurements of methyl acrylate partitioning between water and poly(styrene-*co*-methyl acrylate) latex particles at 45 °C. Theoretical predictions with eq 8 ($\chi = 0.2$ and $\gamma = 45$ dyn·cm^{-1}) (—) and eq 10 (corr = 0.1) (– – –). Data from ref 11.

high-volume fractions of polymer (i.e., at partial swelling). These terms are incorporated into a correction term and calculated from saturation swelling data using the Morton equation.[11]

$$\text{corr} = \chi v_{p,sat}^{\ 2} + \frac{2V_m\gamma v_{p,sat}^{\ 1/3}}{R_oRT} = -\left[\ln(1 - v_{p,sat}) + v_{p,sat}\left(1 - \frac{1}{M_n}\right)\right] \quad (9)$$

This correction term is used in eq 8:

$$\ln(1 - v_p) + v_p\left(1 - \frac{1}{M_n}\right) + \text{corr} = \ln\left(\frac{[M]_{aq}}{[M]_{aq,sat}}\right) \quad (10)$$

The approach used to derive eqs 9 and 10 incorrectly assumes that the both the interfacial free energy and the residual free energy terms are independent of the volume fraction of polymer. However, in the latex particle situation, since the absolute values of these terms are small compared to the configurational entropy term (Figure 1), this approach, as a first estimate, gives quite good results (Figure 2).[11] Note that this approach requires no knowledge of interfacial free energy or the free energy associated with the residual interactions between the solvent and polymer—the correction term can be calculated solely from a knowledge of the saturation concentration of solvent in the latex particles. This latter quantity can be simply determined by experiment.[11]

We now turn to phospholipid vesicles. It is our assertion that simple concepts, similar to those used in the derivation of eqs 8–10, can be utilized to describe the partial swelling of phospholipid vesicles by solvents. One assumption used here is that the mixing of solvent and the alkane part of the phospholipid molecules is well described by Flory–Huggins theory. That is, we treat the alkane part of the phospholipid molecules as a polymer. This will be valid if the alkane chains are immobile relative to the solvent mobility—this ensures that the mixing of the alkane part of the phospholipid chains and solvent can be considered as a volume- or space-filling exercise. This criteria will probably be satisfied since the phospholipid chains will be confined by the head group immobility in the hydrophobic part of the bilayers.[15b] Note that many statistical mechanical models derived for this problem assume that the alkane parts of the phospholipid chains are fixed at the bilayer interface,[7] which, ironically, is the one condition that allows the simple Flory–Huggins theory to be used.

Obviously the degree of saturation swelling of vesicles is limited compared to say a bulk phase. This limitation is due to the free energy associated with the vesicle–water interface and also, in common with swelling in the bulk phase, to the residual free energy of mixing of the solvent and alkane part of the phospholipid bilayer. An equation, analogous to the Morton equation, can be developed for vesicles so long as an expression for the vesicle–water interfacial free energy is available. This is not as simple as it may seem since, instead of one interface in the latex particle case, there are two interfaces in the vesicle situation. Further, these two interfaces have different curvatures. The nature of the surface free energy of vesicles has been discussed in depth by Tanford[16] and also by White.[17]

One result that follows from the considerations of White[17] is that we cannot discern between the contributions from the two interfaces. Tanford[16] asserted that since vesicles are permeable to water the inside and outside pressures of a vesicle must be equal. Tanford then went on to state that this fact causes the macroscopic interfacial tension of a vesicle to be equal to zero. White pointed out that this was not the case: in fact the surface of a vesicle can be at equilibrium while having a non-zero interfacial tension. White gave the following equation for the macroscopic interfacial tension of a vesicle:[17]

$$\gamma = \int_{z_1}^{z_2}[P_N(Z) - P_T(Z)]\,dz \quad (11)$$

where the Z axis is normal to the surface layer and the integration limits Z_1 and Z_2 include the entire bilayer. The interfacial tension, γ, arises from the anisotropy of the bilayer, and the pressure within the bilayer is then a tensor. This pressure has been resolved into two components, $P_N(Z)$ normal to the surface and $P_T(Z)$ tangential to the surface. Note that γ is an integrated property of the interface.

For the vesicle structure there is no single or simple solution for eq 11; hence it is difficult to consider the interfacial tension of the individual monolayers of a bilayer. Therefore, in what follows, we have taken one step back in our considerations. Since we are dealing with large vesicles we can use the Laplace equation to describe the macroscopic surface free energy of vesicles:[16,17]

$$P_o - P_i = \frac{2\gamma}{R_o} \quad (12)$$

where $P_o - P_i$ is the pressure difference across the vesicle, γ is now the macroscopic surface tension of the vesicle, and R_o is the radius of curvature of the vesicle. The use of this equation runs contrary to the statements of White, who states that this equation cannot be used since the radii of the vesicles are similar in size to the vesicle wall thickness. This is not strictly the case for the vesicles discussed in this work. Further, the sizes of the vesicles considered in this work are similar to those of latex particles previously studied in which we have shown that the radii are indeed large enough to use the Laplace equation.[9–14] Most importantly, it is the use of the Laplace equation for vesicles which is being tested in this work—the validity of this will be assessed in the light of comparison

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(17) (a) White, W. H. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 4048. (b) For the purpose of calculations V_p is given by $V_p = {}^4/_3\pi(R_o^3 - R_i^3)v_p$, where R_o is the outer radius of the vesicle bilayer, R_i the inner radius of the vesicle bilayer, and v_p the fraction of phospholipid alkane on the bilayer. V_s is similarly given by $V_s = {}^4/_3\pi(R_o^3 - R_i^3)(1 - v_p)$. The volume of the water interior of the vesicle, V_w, is simply given by $V_w = {}^4/_3\pi R_i^3$.

of theory and experiment, especially in regard to the meaning of the interfacial tension for a bilayer system.

In summary we are going to adopt the form of the Laplace equation for vesicles. The equivalent surface free energy term in eq 5 for vesicles is given by the Gibbs–Thomson equation:

$$\Delta G_s = \frac{2V_m\gamma v_{pv}^{1/3}}{R_o} \tag{13}$$

where R_o is the macroscopic outer radius of the vesicle, V_m is the molar volume of the solvent, γ the macroscopic surface tension of the vesicle, and v_{pv} is the volume fraction of the alkane part of the phospholipid molecules in the whole swollen vesicle. Note that this fraction includes the volume of water in the interior of the vesicle, i.e.

$$v_{pv} = \frac{V_p}{V_p + V_s + V_w} \tag{14}$$

where V is the absolute volume and the subscripts p, s, and w represent, respectively, the alkane part of the phospholipids in the bilayer, the solvent part in the bilayer, and interior water.[17b] The use of this fraction (eq 14) was rationalized by the desire to keep the forms of the equations for vesicles and latex particles the same. The inclusion of water in eq 14 above accounts for the total volume of the sphere that is described by the vesicle.

Note that as a vesicle bilayer is swollen by solvent any change in vesicle radius is due to expansion of the bilayer only, and there is assumed to be no change in volume of the water interior. Hence, the use of the above form of the Gibbs–Thomson equation can be justified since the only change in radius of a vesicle upon swelling results from the absorption of solvent into the bilayer—the change of radius is simply encapsulated in the $v_{pv}^{1/3}$ term.

The full equation describing the swelling of vesicles by solvent is then just given by

$$\ln(1 - v_p) + v_p\left(1 - \frac{1}{M_n}\right) + \chi v_p^2 + \frac{2V_m\gamma v_{pv}^{1/3}}{R_oRT} = \ln\left(\frac{[M]_{aq}}{[M]_{aq,sat}}\right) \tag{15}$$

where v_p is redefined here as the volume fraction of phospholipid alkane in the vesicle bilayers and M_n as one-half the number of carbon chains in the alkane backbone of a single phospholipid chain (this ensures that this term is consistent with the use of an interaction parameter, χ, from polymer-type systems). These redefinitions hold for the rest of this paper.

Note that, at saturation, the left-hand side of eq 15 is equal to zero. The saturation concentration of solvent in a vesicle bilayer can be simply calculated if values for χ and γ are known or can be estimated.

Arguments similar to those used in the derivation of eqs 9 and 10 can be developed for the vesicle situation. At the high-volume fractions of the alkane in the vesicle interior (this just replaces the polymer component in the latex particles), if the dominant contribution to swelling is just given by the configurational entropy of mixing of solvent and the alkane part of the phospholipid, eq 10 can be used to model partial swelling of vesicles, where the correction factor is just given by

$$\text{corr} = \chi v_{p,sat}^2 + \frac{2V_m\gamma v_{pv,sat}^{1/3}}{R_oRT} = -\left[\ln(1 - v_{p,sat}) + v_{p,sat}\left(1 - \frac{1}{M_n}\right)\right] \tag{16}$$

where $v_{p,sat}$ is the volume fraction of phospholipid alkane in the vesicle at saturation swelling by the solvent (redefined for the rest of this paper). Note that eqs 9 and 16 are identical if the second definition of the correction factor is used, i.e. if "corr" is a function of $v_{p,sat}$ and M_n.

Results and Discussions

Experimental results on the partial swelling of multilamellar vesicles by hexane and benzene have been reported by De Young and Dill.[1,2] The phospholipids utilized by these workers are dimyristoylphosphatidylcholine (DMPC) and protiated egg phosphatidylcholine (egg PC). The phospholipid main phase transition temperatures, T_c, of these phospholipid species are $T_c = 23$ °C (DMPC) and $T_c = -10$ °C (egg PC). In what follows we utilize these reported experimental results of multilamellar partial swelling by the solvents hexane and benzene. Note that we only utilize data that are above the phase transition temperature of the phospholipids (swelling below the phase transition temperature is more complex since a ternary solvent system is described up to a solvent fraction of solvent in the vesicles that allows "plasticization" of the phospholipid alkane phase). These data were reported as solvent partition coefficients and as a function of mole fraction of the solvent in the lipid. For comparison of the data with eq 10 we have converted this data to the form shown in Figures 3 and 4, i.e. the natural log of the ratio of the observed aqueous phase concentration of the solvent and the saturation concentration of that solvent in water at the experimental temperature versus the volume fraction of phospholipid alkane in the vesicles.

Dill et al. also reported data[1,2] in which cholesterol was used as a cosolvent in bilayer swelling experiments. Cholesterol acts as a cosolvent (and possibly also as a cosurfactant), and the thermodynamic equations derived specifically for the single solvent case as described in the Theory no longer apply. Recently we have considered swelling of latex particles by two solvents,[13] including the partial swelling situation.[14] The application of these equations to bilayer swelling data with more than one solvent will be published separately.

The use of eq 10 in comparing theory with experiment requires the values of three parameters: the average degree of "polymerization" of the alkane part of the phospholipid chains and the saturation concentrations of the solvent in both the bilayer and aqueous phases. In what follows we discuss each of these parameters separately:

(1) The average degree of "polymerization" of the alkane part of the phospholipid chains, M_n, is defined as one-half the number of backbone carbon atoms in the chain. As stated in the previous section this is simply to make this term consistent with the use of a interaction parameter, χ, in polymer-type systems. In most cases it will be shown that these considerations are of little importance since the configurational entropy term containing M_n contributes negligibly to the thermodynamic equilibrium at the higher values of v_p typically found in vesicles. The values of M_n used in these calculations are listed in Table 1.

(2) The saturation concentration of the solvent in the lipid phase is a difficult parameter to measure directly since saturation of a phospholipid system with solvent

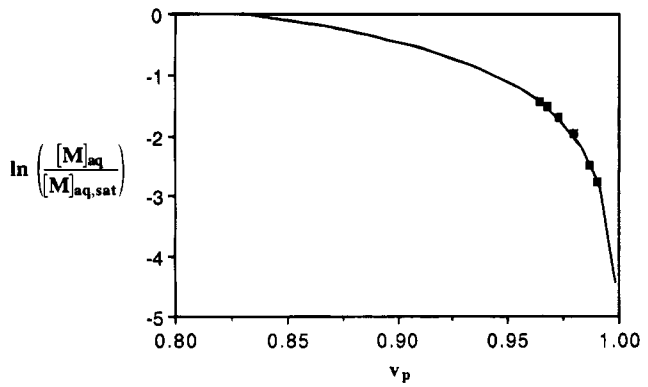

Figure 3. Partial swelling of hexane between the vesicle phase and aqueous phase. Experimental values of $\ln([M]_{aq}/[M]_{aq,sat})$ versus volume fraction of phospholipid alkane in the vesicles for DMPC at 25 °C. The lines represents the fit of eq 10 to experiment with values of $v_{p,sat}$ (in eq 16) as given in Table 2.

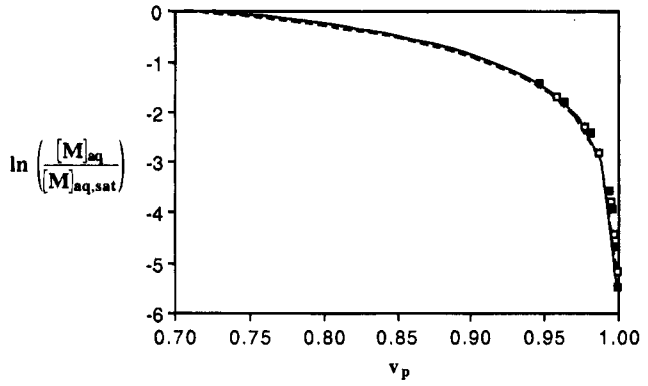

Figure 4. Partial swelling of benzene between the lipid phase and aqueous phase. Experimental values of $\ln([M]_{aq}/[M]_{aq,sat})$ versus volume fraction of phospholipid alkane in the vesicles for DMPC at 30 °C (full squares) and egg PC at 25 °C (empty squares). The lines are fits of eq 10 to experiment for DMPC at 30 °C (full line) and egg PC at 25 °C (dashed line) with values of $v_{p,sat}$ (in eq 16) as given in Table 2.

Table 1. Parameters Utilized in the Fitting of Eqs 10, 15, and 16 to Experimental Partitioning Data

parameter	value	ref
$[benzene]_{aq,sat}$ at 20 °C	2.28×10^{-2} mol·dm^{-3}	19
$[hexane]_{aq,sat}$ at 20 °C	1.28×10^{-4} mol·dm^{-3}	19
mol vol of benzene at 20 °C	88.7 mL·mol^{-1}	19
mol vol of hexane at 20 °C	130.7 mL·mol^{-1}	19
mol vol of water	18.07 mL·mol^{-1} at 25 °C 18.10 mL·mol^{-1} at 30 °C	20
specific vol of DMPC	0.933 mL·g^{-1} at 10 °C 0.970 mL·g^{-1} at 25 °C 0.973 mL·g^{-1} at 30 °C	21
specific vol of egg PC	0.970 mL·g^{-1} at 25 °C	estd from ref 22
mol mass of DMPC	707 g·mol^{-1}	
mol mass of egg PC	733 g·mol^{-1}	
M_n of DMPC	7	
M_n of egg PC	7	
χ benzene–polyisoprene 25–50 °C	0.45	23
χ hexane–polyisoprene 25–50 °C	0.52	23
R_o	50, 400 nm	
thickness of vesicle bilayer	3 nm	

may lead to solvent droplet formation, especially when the mixture is stirred. Therefore, in the absence of measured saturation concentrations of solvent in the vesicles, in comparing eq 10 with experimental results, we treat the correction term (eq 16) as an adjustable parameter, or more properly, the saturation volume fraction of phospholipid alkane in the vesicles, $v_{p,sat}$, is treated as an adjustable parameter. The ramifications of this will be discussed below.

Table 2. Fitted Values of the Correction Factor in Eq 16, Volume Fraction of Alkane in the Vesicles at Saturation, Concentration of Solvent in the Vesicles at Saturation, Solvent Partition Coefficient at Saturation, Solvent Partition Coefficient at Infinite Dilution, and Vesicle Interfacial Tension at Saturation

	vesicle type		
parameter	DMPC	DMPC	egg PC
solvent	hexane	benzene	benzene
T (°C)	25	30	25
corr	1.04	0.65	0.60
$v_{p,sat}$	0.82	0.72	0.70
$[M]_{v,sat}$ (mol·dm^{-3})	1.4	3.2	3.4
k_∞	3.4×10^5	4.1×10^3	4.5×10^3
K_{sat}	2.3×10^5	1.8×10^3	1.9×10^3
γ (dyn·cm^{-1})	25–50	25–50	18–37

(3) The water solubilities of the solvents are readily found in the literature. Note that, at all temperatures, single values of the water solubilities were utilized in the calculations. This was shown to introduce an imperceptible error over the small temperature range at which the experiments were carried out.

In Figures 3 and 4 the fits of eq 10 to experiment are displayed. In all cases a very good fit is observed. The only adjustable parameter in these fits is the volume fraction of phospholipid alkane at saturation swelling by the solvent ($v_{p,sat}$), which arises from the use of eq 16 with eq 10. The value of this parameter is constrained by two factors. First, $v_{p,sat}$ must be less than all the partial saturation volume fractions, and secondly, $v_{p,sat}$ must be greater than zero, probably with a value typical for micelles and very small latex particles. It cannot be too small nor too large. The fitted values of $v_{p,sat}$ are shown in Table 2. From the values of $v_{p,sat}$ the saturation partition coefficients, K_{sat}, can be calculated from

$$K_{sat} = \frac{[M]_{v,sat}}{[M]_{aq,sat}} \tag{17}$$

where $[M]_{v,sat}$ is the saturated concentration of solvent in the vesicles. Values of $[M]_{v,sat}$ and K_{sat} are displayed in Table 2. Values for maximum solvent uptake by other surfactant structures, e.g. benzene into sodium dodecyl sulfate micelles ($[M]_{micelle,sat} = 2.5$ M, $K_{sat} = 1.6 \times 10^3$),[18] are about the same order of magnitude. Similar values are found for small (radii < 20 nm) latex particles.[10] Values of K_{sat} are all less than the bulk values (at 25 °C, $K_{sat,hexane} = 4.5 \times 10^5$ and $K_{sat,benzene} = 2.4 \times 10^3$).[19] This is expected since the degree of solvent uptake by vesicles is restricted by the interfacial free energy. The fitted values of $[M]_{v,sat}$ and K_{sat} all compare well with expectations.

In Figures 5 and 6 the raw partitioning data are compared to the fits of eq 10, i.e partition coefficient versus mole fraction of solvent in the lipid. In all case a good fit is achieved. Notice that the fitted curves are not exactly linear, as has often been assumed.

The value of the solvent partition coefficient at infinite dilution, K_∞, was calculated from extrapolation of the fitted lines in Figures 5 and 6 to zero mole fraction of solvent.

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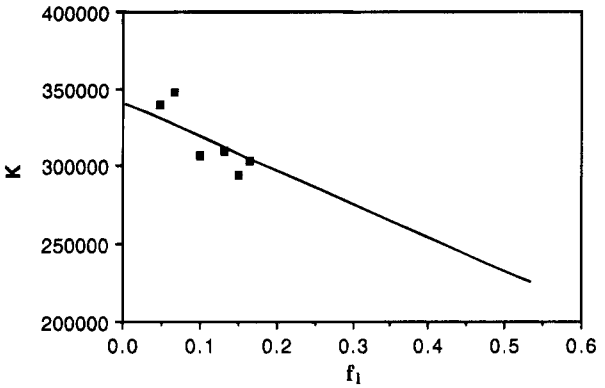

Figure 5. Partial swelling of hexane between the vesicle phase and aqueous phase. Experimental values of partition coefficient versus fraction of hexane in the lipid phase for DMPC at 25 °C. The line represents the fit of eq 10 to experiment with values of $v_{p,sat}$ (in eq 16) as given in Table 2.

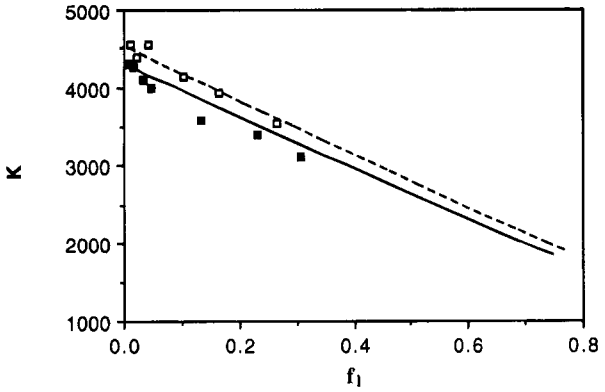

Figure 6. Partial swelling of benzene between the lipid phase and aqueous phase. Experimental values of partition coefficient versus fraction of benzene in the lipid phase for DMPC at 30 °C (full squares) and egg PC at 25 °C (empty squares). The lines are fits of eq 10 to experiment for DMPC at 30 °C (full line) and egg PC at 25 °C (dashed line) with values of $v_{p,sat}$ (in eq 16) as given in Table 2.

The excellent agreement between theory and experiment for a range of solvents, phospholipid types, and temperatures suggests that the mixing of solvent and the alkane part of phospholipid chains is adequately described by Flory–Huggins theory. It is known[11] that, at high-volume fractions of the polymer in latex systems, the dominant free energy contribution to the equilibrium situation is the configurational entropy of mixing of solvent and the alkane chains. The good agreement between theory and experiment in this work implies that at partial swelling the nonconfigurational and enthalpic terms are small compared to the configurational entropy term. More surprising, since vesicles are considered as interfacial phases of matter, is that the interfacial free energy is relatively small and constant (with respect to the configurational entropy free energy term) at partial swelling. Upon further reflection the cause for this result can be easily explained: the changes of the surface area with swelling over the full range of swelling is small. This is simply because the vesicles do not swell to a very large extent. Hence, relative to the configurational entropy of mixing the interfacial free energy at partial swelling does not contribute significantly to *changes* in the thermodynamic equilibrium attained at various degrees of swelling by solvent. This is displayed in Figure 7.

It has been noted that the solvent partition coefficient changes with the surface density of phospholipid in the vesicles (i.e., the area per phospholipid molecule on the surface of the vesicles).[2] Obviously, as more solvent is

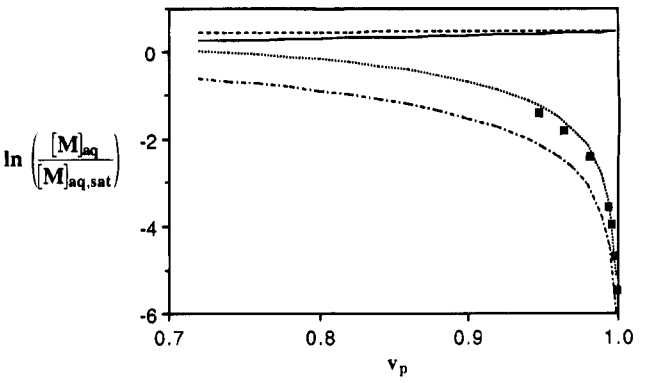

Figure 7. Comparison of theoretical predictions (assuming χ and γ are independent of v_p) and experimental measurements of benzene partitioning between water and DMPC vesicles at 30 °C. Theoretical predictions: configurational entropy term given by $\ln([M]_{aq}/[M]_{aq,sat}) = \ln(1 - v_p) + v_p(1 - 1/M_n)$ (– · –); residual free energy term given by $\ln([M]_{aq}/[M]_{aq,sat}) = \chi v_p^2$, with $\chi = 0.45$ (—); interfacial free energy term given by $\ln([M]_{aq}/[M]_{aq,sat}) = 2V_m\gamma v_{pv}^{1/3}/R_oRT$, with $\gamma = 13.4$ dyn·cm^{-1} and $R_o = 50$ nm (– – –); and eq 15 with $\chi = 0.45$, $\gamma = 25$ dyn·cm^{-1}, and $R_o = 50$ nm (• • •).

imbibed by the vesicles, the surface density will decrease, simply because the phospholipid head groups are slightly further apart. This correlation does not necessarily imply that surface density is the dominating factor that limits the degree of swelling. The configurational entropy, and not the surface free energy, appears to dominate the changes in the swelling behavior at various degrees of saturation of vesicles by solvent.

At saturation swelling it is true that the degree of swelling is limited, to a large degree by the interfacial free energy. It was shown above that the values for saturation swelling of vesicles by solvents can be compared to those for micelles, or very small latex particles, where the large surface area to volume ratio restricts swelling to far below the bulk value. It must be noted that this does not imply that mixing of the alkane part of the phospholipids and solvent *cannot* be described by bulk thermodynamic expressions: to the contrary, the latter must only be combined with the appropriate interfacial free energy terms.

Note that, if vesicles are swollen to a degree greater than the "saturation" values described in this work, it is likely that the phospholipid molecules on either side of a single bilayer will be separated, either in full or in part, by a region of pure solvent. In these cases the theory developed in this work does not apply, and the vesicle should properly be considered as a type of complex solvent droplet. Whether there is a true thermodynamic "saturation" concentration of solvent in vesicle bilayers is a complex issue which may be confused by kinetic effects in experiments. We will discuss this issue in a future publication.

If we know the vesicle thickness, vesicle radius, and the interaction parameter between the solvent and alkane chains of the phospholipid molecules then, from the experimentally fitted values of $v_{p,sat}$ and eq 16, the value of the vesicle–water interfacial tension can be determined. However, the former three parameters warrant further comment:

(1) Bilayers of the type in this work are known to have a thickness of between 2 and 4 nm. This is related to the length of the phospholipid molecules. In this work we utilize a mean thickness of 3 nm.

(2) Multilamellar vesicles are known to be from 50 to 400 nm in radius. In this work we have calculated values at both 50 and 400 nm.

(3) The interaction parameter of the solvents with the alkane chain of the phospholipids can be approximated by the interaction parameter between the solvent and polyisoprene. These interaction parameters measured between the solvents and polyisoprene represent the most similar (measured) system to the vesicle situation at hand, namely alkyl tails of surfactants mixing with the same solvents. Polyisoprene is an alkyl polymer with some degree of unsaturation and a methyl side group on the main chain, whereas the alkyl chains of the phospholipid molecules are generally unsubstituted and contain a small degree of unsaturation. It should be noted that these differences in structure are unlikely to significantly affect the value of the interaction parameter. Using this approximation for the interaction parameters ignores the possible interactions of solvent molecules with the head groups of the phospholipids (this is consistent with the approach of this work). At 25–50 °C the measured interaction parameters between polyisoprene and benzene and hexane, respectively, are $\chi = 0.45$ and $\chi = 0.52$.

Utilizing the above values of the bilayer thickness, χ and R_o, from the values of $v_{p,sat}$ calculated by fits of eq 10 to experimental data, it is possible, via eq 16, to estimate the interfacial tension of the vesicles. These interfacial tensions are given in Table 2. The range of interfacial tensions are those expected and are well below the water–solvent interfacial tensions (of course the exact values of the interfacial tension rely upon better values for the interaction parameters). Of greater interest is that the interfacial tensions of the hexane–DMPC and benzene–DMPC data are very similar despite the large differences in their swelling behavior. This is encouraging since if the interfacial tension is truly a interfacial phenomena then solvent should have little effect on this value since the surface is mainly characterized by surfactant (this is because of the low solvent concentrations in vesicles). This appears to be the case.

Utilizing the above calculated interfacial tensions, in Figure 7, the contributions of all terms in eq 15 to the equilibrium partitioning of benzene in DMPC vesicles are displayed. In accord with the assumptions made, it can be clearly seen that, at partial swelling, the dominant contribution to the changing thermodynamic equilibrium is the configurational entropy of mixing of solvent and the alkane part of the phospholipid molecules.

Conclusions

Comparison of theory and experiment suggests that the swelling of vesicles by solvents can be modeled in a manner similar to that for the swelling of polymer latex particles by solvent. Data for the swelling of various bilayers by two solvents at two temperatures were all adequately fitted by the model. The only "adjustable" parameter in this model, the volume fraction of solvent or alkyl chain in the vesicle at saturation, is in fact limited by considerable physical understanding: the fitted values of this parameter compare well to values for similar systems.

The basis of the equations utilized in this paper is the consideration that the mixing of the alkyl part of the vesicles and solvent can be considered in terms of Flory–Huggins theory. The Flory–Huggins theory works very well for the partial swelling of vesicles with solvent for the following reasons.

(1) The alkane concentrations within the vesicles are always relatively high. Therefore there should be a uniform density of Flory–Huggins segments.

(2) The residual free energy term (the χ term) is not a major contributor to the thermodynamic problem at the higher volume fraction of alkane. Hence all the uncertainties associated with the measurement and interpretation of the interaction parameter are less consequential. As pointed out by Flory the original derivation of the Flory–Huggins theory resulted in an interaction parameter that should be polymer (phospholipid alkane) concentration dependent at high-volume fractions of polymer (phospholipid alkane). This is not a problem for the experimental systems studied in this work since within experimental error the fit of eq 10 to experimental data was insensitive to the value of the interaction parameter (within reasonable bounds). The Flory–Huggins term that describes the configurational entropy of mixing of solvent and polymer is very successful at predicting the solvent partitioning. This entropic term was also derived by Hildebrand via a free volume approach and is quite general.

(3) The alkyl tails of the phospholipid chains are relatively immobile compared to solvent molecules, and therefore, the phospholipid molecules can be considered as fixed in space. In this case the placement of solvent molecules in the vesicles becomes a free volume problem, which is that which the Flory–Huggins approach addresses.

The interfacial free energy of vesicles was modeled with the Gibbs–Thomson equation, utilizing a single macroscopic interfacial tension. From the fit of the model to the experimental data, and specifically from the fitted value of the saturation concentration of solvent in the vesicles (here both γ and χ are important), a range of values of the interfacial tension of the vesicles were calculated. These values are similar to those expected for small latex particles and micelles.

Unfortunately, the use of a single interfacial tension tells us very little about the individual interfaces of the bilayer, except that, being non-zero, it confirms White's assertion that a vesicle can be at equilibrium even if the interfacial tension is non-zero: this just arises from the proper definition of the interfacial tension (eq 11). The use of a macroscopic interfacial tension is consistent with the use of bulk thermodynamic expressions for the hydrophobic part of the bilayers. Now that we have shown that these equations can indeed describe certain vesicle behavior the next challenge will be to relate these macroscopic properties to the microscopic.

Finally, it was the original aim of this work to develop a relationship that could model the swelling of vesicle bilayers by solvent. The equations that are developed here can be used to predict the swelling of vesicles by solvent and require that only the saturation concentration of the solvent in the vesicle be known. If this is known, an estimate of the interfacial tension of the vesicle can also be calculated (or vice versa). The success of this work may allow for the modeling of solvent uptake by other types of bilayers (e.g., extended sheets), and even other surfactant structures (e.g., micelles), since the mixing of the solvent and surfactant within the interior of the vesicles can be easily modeled by macroscopic thermodynamic theory. This latter point may have farther reaching ramifications.

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