Shift in membrane miscibility transition temperature upon addition of short-chain alcohols

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(Received 18 August 2016; published 12 December 2016)

I consider the effect of a small concentration of a molecule, such as a short-chain alcohol, on the miscibility transition temperature of a giant plasma membrane vesicle. For concentrations sufficiently small such that the system can be treated as a dilute solution, the change in transition temperature is known to depend upon the extent of the molecule's partition into the coexisting liquid-disordered and liquid-ordered phases. Preferential partitioning into the former decreases the miscibility temperature, while preferential partitioning into the latter causes an increase. The analysis, combined with calculated values of the partition coefficient of saturated chains, illuminates the results of recent experiments on the change in miscibility transition temperatures with changing alcohol chain length, and makes several testable predictions.

DOI: 10.1103/PhysRevE.94.062114

I. INTRODUCTION

It is well known that model membranes, consisting of a ternary mixture of saturated lipids, unsaturated lipids, and cholesterol, exhibit a liquid-liquid miscibility phase transition [1]. The two liquid phases are distinguished by their composition and the extent of the variation, in the lipids' acyl chains, of the angle between adjacent carbon-carbon bonds and the membrane normal. The phase with the greater variation is denoted [2] liquid-disordered (ld). It is rich in unsaturated lipid. The phase with the lesser disorder is denoted [2] liquid-ordered (lo), and is rich in saturated lipids. Both phases are characterized by a diffusion constant typical of a two-dimensional fluid, as opposed to the much smaller one of the more ordered, and more dense, gel phase. Complex, cell-derived. giant plasma membrane vesicles also exhibit such a liquid-liquid transition [3].

It has recently been shown that the introduction of short-chain alcohols into cell-derived giant plasma membrane vesicles affects the temperature of transition from a single, macroscopically uniform phase, to coexisting lo and ld phases [4]. The miscibility transition temperature decreases on the introduction of ethanol. If the length of the chain in the n-alcohols is made larger, the magnitude of the change in temperature increases through propanol, octanol, and decanol. With further increase in n, however, that trend reverses, and the magnitude decreases such that tetradecanol ($n = 14$) exhibits no effect on the transition temperature. This behavior is interesting in light of the result that the introduction of cholesterol into a giant unilamellar vesicle consisting of a mixture of two miscible lipids causes them to undergo phase separation, that is, it *increases* the miscibility transition temperature [5]. The results of Gray *et al.* [4] are not without precedent, however, as it was observed long ago that alcohols with n less than 12 depress the gel-liquid transition temperature [6]. The observed behavior was interpreted in terms of a thermodynamic result for a dilute solution, a result, derived below, that relates the temperature shift to the partitioning of the alcohol between the liquid and gel phase.

It is the purpose of this paper to show that if the alcohol forms a dilute solution in the membrane, then the change in the temperature of a first-order miscibility transition exhibits the same behavior with alcohol chain length as that observed by Gray *et al.* [4]. To show this, I utilize a simple thermodynamic argument [7] and the results of a recent calculation of the partition coefficients of single chains in coexisting lo and ld phases [8]. This combination makes several testable predictions about the temperature change that would be observed were longer-chain alcohols to be employed, or larger concentrations of shorter-chain alcohols to be introduced. I also emphasize that the change in the temperature of a miscibility transition upon the introduction of an alcohol into a membrane containing p components is not a well-defined quantity unless the behavior of the other $p + 1$ independent thermodynamic variables is specified.

II. THERMODYNAMICS

I first review the argument of Landau and Lifshitz [7] concerning the change in the temperature of a first-order transition upon the introduction of a solute into a one-component membrane acting as a solvent. In the absence of solute, the internal energy of a bilayer with entropy S, number of solvent particles N, and area A is given by

$$U = TS + \sigma A + \mu N, \tag{1}$$

with differential

$$dU = TdS + \sigma dA + \mu dN, \tag{2}$$

where T, σ, and μ are the temperature, surface tension, and chemical potential, respectively. Differentiating the first equation above and comparing with the second, one obtains the Gibbs-Duhem relation

$$SdT + Ad\sigma + Nd\mu = 0. \tag{3}$$

A convenient thermodynamic potential for the system is the Gibbs free energy

$$\Phi_0(T,\sigma,N) = U - TS - \sigma A = N\mu_0(T,\sigma). \tag{4}$$

The potential can be calculated from the partition function

$$\begin{aligned} Q_0(T,\sigma,N) &= \exp[-\Phi_0(T,\sigma,N)/k_BT] \\ &= \frac{1}{N!}\mathrm{Tr}\,\exp\{-[H(N,A) - \sigma A]/k_BT\}, \end{aligned} \tag{5}$$

where H is the Hamiltonian of the system. Now let n_s molecules of solute be added to the system and consider the effect on the thermodynamic potential, which becomes $\Phi(T,\sigma,N,n_s)$. Because the n_s solute particles are indistinguishable, the partition function becomes

$$\begin{aligned} Q(T,\sigma,N,n_s) &= \exp[-\Phi(T,\sigma,N,n_s)/k_BT] \\ &= \frac{1}{N!n_s!}\mathrm{Tr}\,\exp\{-[H(N,n_s,A)-\sigma A]/k_BT\}, \end{aligned} \tag{6}$$

so that

$$\begin{aligned} \Phi(T,\sigma,N,n_s) = \Phi_0(T,\sigma,N) &+ n_sk_BT\,\ln(n_s/e) \\ &- k_BT\,\ln\left[\frac{n_s!Q(T,\sigma,N,n_s)}{Q_0(T,\sigma,N)}\right], \end{aligned} \tag{7}$$

where Stirling's approximation has been used. Further the thermodynamic potential must be a homogeneous function of N and n_s of order one, i.e.,

$$\Phi(T,\sigma,\lambda N,\lambda n_s) = \lambda\Phi(T,\sigma,N,n_s), \tag{8}$$

for arbitrary λ. From Eqs. (4), (7), and (8) it can seen that for a weak, or dilute, solution, one for which $n_s \ll N$, the thermodynamic potential must have the form, to first order in n_s,

$$\begin{aligned} \Phi(T,\sigma,N,n_s) = N\mu_0(T,\sigma) &+ n_sk_BT\,\ln(n_s/eN) \\ &+ n_s\psi(\sigma,T), \end{aligned} \tag{9}$$

where the function ψ depends only on σ and T. The first term is the potential in the absence of solute. The form of the second term, the entropy of mixing, follows from the fact that Eq. (8) requires that the logarithm depend on the ratio n_s/N; the third term from the fact that with the extensivity appearing directly in n_s, any function that it multiplies can depend only on powers of n_s/N, which would contribute to Φ terms of higher order in n_s, and on σ and T.

From Eq. (9) it follows that the chemical potential of the solvent is, to first order in the solute mol fraction, or concentration $c \equiv n_s/N$, given by

$$\mu(T,\sigma,c) = \frac{\partial\Phi(T,\sigma,N,n_s)}{\partial N} = \mu_0(T,\sigma) - k_BTc. \tag{10}$$

Note that this change in the solvent chemical potential arises solely from the entropy of the solute. Contributions to the solvent chemical potential from interactions between solute molecules and other molecules, solvent or solute, are of higher order in the solute concentration.

Consider a first-order transition from one uniform phase to two coexisting phases, denoted I and II. In the case of a pure one-component solvent, the condition for coexistence is that, in addition to the temperature and surface tension of each phase being equal, the thermodynamic potentials, or equivalently the chemical potentials, of each phase must also be equal:

$$\mu_0^I(T_{0,co},\sigma_0) = \mu_0^{II}(T_{0,co},\sigma_0). \tag{11}$$

This condition determines the coexistence curve $T_{0,co} = T_{0,co}(\sigma_0)$. The transition temperature is completely determined by the surface tension of the two coexisting phases.

With the addition of a solute forming a dilute solution, the condition of the equality of solvent chemical potentials becomes, from Eq. (10)

$$\mu_0^I(T,\sigma) - c_Ik_BT = \mu_0^{II}(T,\sigma) - c_{II}k_BT. \tag{12}$$

The change in transition temperature on the addition of solute is obtained by expanding $\mu_0(T,\sigma)$ about $\mu_0(T_{0,co},\sigma_0)$. Denoting $T = T_{0,co} + \Delta T$ and $\sigma = \sigma_0 + \Delta\sigma$ and utilizing Eq. (3) from which $\partial\mu_0/\partial T = -S/N \equiv -s$, $\partial\mu_0/\partial\sigma = -A/N \equiv -a$, one obtains

$$\Delta T = -\frac{a_I - a_{II}}{s_I - s_{II}}\Delta\sigma - \frac{c_I - c_{II}}{s_I - s_{II}}k_BT. \tag{13}$$

Note that the coexistence temperature in the dilute solution is no longer determined by the surface tension alone, but by the amount of solute as well. That is, the coexistence line of the pure solvent in the T,σ plane is, for the solution, drawn out into a sheet in the space of T,σ and μ_s, the solute chemical potential. Thus the change in transition temperature ΔT upon the addition of solute is only a meaningful quantity when the change, if any, of the independent thermodynamic variable, the surface tension, is specified. For example, the miscibility transition temperature has been intentionally varied by controlling the surface tension explicitly [9]. In the case in which the surface tension is held fixed, Eq. (13) reduces to

$$\Delta T = -\frac{c_I - c_{II}}{s_I - s_{II}}k_BT. \tag{14}$$

The equation explains, *inter alia*, the observation [10] that the addition of cholesterol to a one component membrane at constant tension causes a decrease in the transition temperature from liquid to gel phase. This follows because the cholesterol preferentially partitions into the liquid phase [10] which has a larger entropy per particle than does the gel phase [10].

The extension of the result of Eq. (13) to a membrane of p components that acts as a solvent for the solute is straightforward. Let the membrane without solute have N molecules of which $N_i = Nx_i$ are of component $i = 1\ldots p$. The total energy of any given phase can be written

$$\begin{aligned} U &= TS + \sigma A + \sum_{i=1}^{p}\mu_iN_i \\ &= TS + \sigma A + \sum_{i=1}^{p-1}(\mu_i - \mu_p)N_i + N\mu_p, \end{aligned} \tag{15}$$

$$\begin{aligned} \text{with differential}\quad dU = TdS + \sigma dA &+ \sum_{i=1}^{p-1}(\mu_i - \mu_p)dN_i \\ &+ \mu_pdN, \end{aligned} \tag{16}$$

which leads to the Gibbs-Duhem equation

$$SdT + Ad\sigma + N\sum_{i=1}^{p-1}x_id(\mu_i - \mu_p) + Nd\mu_p = 0. \tag{17}$$

I again consider the thermodynamic potential

$$\Phi(T,\sigma,\{N_i\},N) = U - TS - \sigma A,$$

where $\{N_i\}$ denotes the set of $N_i, i = 1, p-1$. In the absence of solute,

$$\Phi_0(T,\sigma,\{N_i\},N) = \sum_{i=1}^{p-1} N_i(\mu_{i,0} - \mu_{p,0}) + N\mu_{p,0}, \quad (18)$$

$$d\Phi_0 = -SdT - Ad\sigma + \sum_{i=1}^{p-1}(\mu_{i,0} - \mu_{p,0})dN_i + \mu_{p,0}dN, \quad (19)$$

and Φ_0 is obtained from the partition function

$$\exp[-\Phi_0/k_BT] = \mathrm{Tr}\prod_{i=1}^{p}\frac{1}{N_i!}\exp\{-[(H-\sigma A)/k_BT]\}, \quad (20)$$

where H is the Hamiltonian of the multicomponent system.

Again, let n_s solute molecules be added to the system changing the thermodynamic potential to $\Phi(T,\sigma,\{N_i\},N,n_s)$. Employing the same arguments as before for a dilute solution, one finds that the chemical potential

$$\mu_p = \frac{\partial\Phi(T,\sigma,\{N_i\},N,n_s)}{\partial N} = \mu_{p,0} - k_BTc. \quad (21)$$

As there is nothing distinguishing the component p, this is true for the chemical potentials of all components.

At coexistence of two phases, the chemical potentials of all components must be equal. It is convenient to consider μ_p a function of T, σ and the $p-1$ independent chemical potential differences $\delta\mu_i \equiv \mu_i - \mu_p$. Then the condition of coexistence can be written

$$\mu^I_{p,0}(T,\sigma,\{\delta\mu_i\}) - k_BTc^I = \mu^{II}_{p,0}(T,\sigma,\{\delta\mu_i\}) - k_BTc^{II}. \quad (22)$$

Assume that, in the absence of solute, the two phases are in coexistence so that

$$\mu^I_{p,0}(T_{0,co},\sigma_0,\{\delta\mu_{i,0}\}) = \mu^{II}_{p,0}(T_{0,co},\sigma_0,\{\delta\mu_{i,0}\}). \quad (23)$$

Now expand the temperature T about $T_{0,co}$, the surface tension σ about σ_0, and the chemical potential differences $\delta\mu_i$ about $\delta\mu_{i,0}$ to obtain the extension of Eq. (13),

$$\Delta T = -\frac{1}{s^I - s^{II}}\left\{(a^I - a^{II})\Delta\sigma + \sum_{i=1}^{p-1}\left(x_i^I - x_i^{II}\right)\Delta(\mu_i - \mu_p) + k_BT(c^I - c^{II})\right\}. \quad (24)$$

In the above $a^I - a^{II}$ is the difference in area per particle of the coexisting phases, $x_i^I - x_i^{II}$ the difference in mol fractions of component i in the coexisting phases, and $c^I - c^{II}$ the difference in the mol fraction of the solute in the coexisting phases. Note that the coexistence temperature is now a function of $p+1$ fields; σ, the $p-1$ chemical potential differences $\{\mu_i - \mu_p\}$, and μ_s the solute chemical potential. These fields, or an equivalent number of conditions, must all be specified if the change in transition temperature upon the addition of solute is to be a meaningful quantity.

The contribution to $\Delta T/T$ from the last term can be written

$$-\frac{k_B}{s^I - s^{II}}(c^I - c^{II}) = -\frac{k_B}{s^I - s^{II}}2\bar{c}\frac{1 - c^{II}/c^I}{1 + c^{II}/c^I} = -\frac{k_B}{s^I - s^{II}}2\bar{c}\frac{1 - X^{II}/X^I}{1 + X^{II}/X^I} \approx -\frac{k_B}{s^I - s^{II}}\bar{c}(1 - X^{II}/X^I), \quad (25)$$

where $\bar{c}$ is the average solute concentration, and X^I and X^{II} are the mol fractions of the solute in the two phases. The last line follows when these mol fractions are not too different from one another.

Let phase I be the liquid-disordered phase and II be the liquid-ordered phase, in which case the entropy difference $s^I - s^{II}$ is positive (see below). Then this contribution to $\Delta T/T$ is negative when the ratio X^{II}/X^I is less than unity and is positive otherwise. The partition coefficient X^{lo}/X^{ld} of several different kinds of chains in a bilayer consisting of dipalmitoyl phosphatidylcholine (DPPC), dioleoyl phosphatidylcholine (DOPC), and cholesterol were obtained recently [8] from a self-consistent-field theory calculation, one that employed over 10^8 configurations of each species of molecule in order to obtain partition functions. Figure 1, reproduced from that paper, shows results that are relevant here. The partition coefficients are plotted as a function of chain length, n. Note that, for saturated chains, the partition coefficient decreases with increasing n for small n, but for n beyond 12 it increases with increasing n and crosses unity for n of about 16. The behavior is not difficult to understand. A saturated chain shorter than those which make up the bilayer partitions preferentially to the liquid disordered phase because its entropy is greater there [11]. This contribution dominates the energetic one which favors the liquid ordered phase. As n increases to that of the saturated chains in the bilayer, the partition coefficient must take a value essentially equal to that of those chains. This follows from the fact that, if one added a lipid which was identical to one of the components of the bilayer, its partitioning into the two phases would simply be obtained from the end points of the tie line connecting them. As saturated chains are found predominantly in the liquid

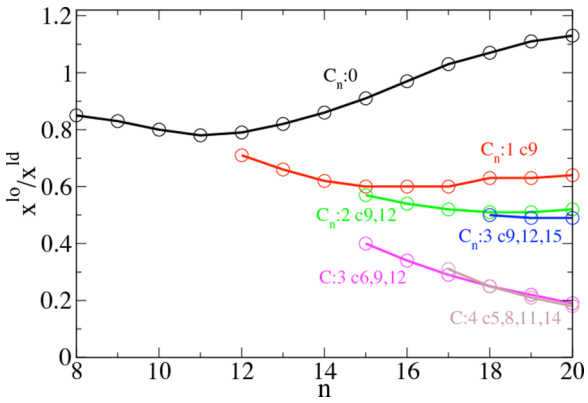

FIG. 1. Partition coefficient, X^{lo}/X^{ld}, for several kinds of single chains of length n. C_n : 0 denotes a chain of length n and no double bonds. From Ref. [8].

ordered phase, the partition coefficient must exceed unity. As a consequence of this behavior of the partition coefficient, the contribution of the last term in Eq. (24) would tend to cause the transition temperature to decrease upon the addition of octonal, and to decrease even more on the addition of decanol. But upon further increase of the chain length, the magnitude of the decrease in transition temperature would become smaller, and eventually vanish. This dependence of transition temperature on chain length is just the behavior observed by Gray *et al.* [4].

III. DISCUSSION

I have shown that, in small concentrations, the addition of a short-chain alcohol to a membrane undergoing a first-order transition to coexisting liquid-ordered (lo) and liquid-disordered (ld) phases causes a change in the transition temperature, as given by Eq. (24); that of the several contributions to the change in transition temperature, one is proportional to the partitioning of the alcohol in the two phases; and that a recent calculation [8] of this partitioning shows that this contribution would cause just the interesting behavior in the temperature shift as a function of chain length as is observed in experiment [4]. Further, I now show that this term, and the observed order of magnitude of shift in the transition temperature, yields a reasonable difference in partitioning of the alcohol. To do so, I need the difference in entropy between ld and lo phases. This can be estimated from a combination of the Clausius-Clapeyron equation,

$$\left.\frac{dT}{d\sigma}\right|_{co} = -\frac{a^I - a^{II}}{s^I - s^{II}}, \tag{26}$$

which gives the change in transition temperature with a change in surface tension, all other thermodynamic variables being fixed, the measured [9] rate of change of transition temperature with surface tension, $dT/d\sigma|_{co} \approx -2.8$ K/mN/m, and a difference in area per particle [12,13] of 0.2 nm^2. This yields a difference in entropy per particle of $(s^I - s^{II})/k_B \approx 5.2$. With this and a measured [4] fractional decrease in transition temperature $\Delta T/T$ of about -0.013, one obtains from the last term of Eq. (24) a value $c^I - c^{II} \approx 0.1$ which is reasonable.

Note that the magnitude of the temperature shift given by Eq. (24) depends upon the nonzero difference in entropy per particle in the two coexisting phases. Hence a calculation which assumes that this difference in entropy is zero, as is in a simple Ising model in which the entropy difference vanishes by symmetry [14], cannot capture this temperature shift in such a dilute solution.

I now address the question as to whether the contribution to the shift in transition temperature due to the partitioning of the solute, the last term in Eq. (24), is the dominant one. The first term in Eq. (24) can certainly be ignored compared to the last for the case of a biological membrane. The change in area per lipid [12,13] between liquid-ordered and liquid-disordered phases is about $\Delta a = 0.2$ nm^2. Further, the surface tension decreases on the addition of solute, and this decrease cannot be larger than the surface tension itself. In cells [15], this is on the order of $5 \times 10^{-3}\ k_BT$/nm^2. Thus in order for the first term in Eq. (24) to be greater than the last, the difference in mol fractions of the solute in the two phases would have to be less than 1×10^{-3}.

There remains to discuss only the terms in Eq. (24) proportional to changes in chemical potential differences $\Delta(\mu_i - \mu_p)$, $i = 1, \ldots, p-1$. It would appear that these quantities are not controlled in the experiment, and to this extent, the change in transition temperature upon addition of alcohol is not a well-defined quantity; i.e., by varying these chemical potentials upon addition of the alcohol, one could vary the shift in transition temperature at will. Nevertheless, it is reasonable to assume that, except for the addition of the short-chain alcohol, the composition of the giant plasma membrane vesicles utilized by Gray *et al.* [4] are essentially the same as vesicles without alcohol. Therefore, with the exception of the change in chemical potential of all solvent components brought about by the entropy of the solute, Eq. (21), a change which does not affect the chemical potential differences $\mu_i - \mu_p$, the chemical potentials are otherwise unaffected. Thus the shifts, $\Delta(\mu_i - \mu_p)$, vanish. If this is the case, then Eq. (24) reduces to

$$\Delta T = -\frac{k_BT(c^I - c^{II})}{s^I - s^{II}}. \tag{27}$$

The above calculation has determined the shift, on the addition of solute, to an onset temperature of transition from a single phase to a region of two-phase coexistence, as in the experiments of Gray *et al.* [4]. I now briefly discuss the case in which there can be more than a single temperature of transition to consider. This situation is most simply discussed in the context of the liquid-gel transition in a one-component membrane. Were the surface tension to be held constant while the temperature of the system in the liquid phase was reduced, then the system would enter the region of two-phase coexistence at a certain temperature; the transfer of liquid phase to gel phase would occur at the same temperature, and the system would emerge from the region of two-phase coexistence at this temperature. The effect of adding a solute, such as cholesterol, on this transition temperature could then be calculated from Eq. (14) and the shift would be unambiguous. However, were the area, rather than the surface tension, to be fixed, then the system in the liquid phase would enter the two-phase region at a certain temperature, T_1, and the temperature would decrease while liquid phase was being converted to gel. Finally, the system would emerge from the coexistence region and become pure gel at a temperature, T_2, lower than T_1. In this case the chemical potentials, $\mu_0(T_1,\sigma_1)$ and $\mu_0(T_2,\sigma_2)$, of the system would differ. Therefore, upon the addition of cholesterol, the shifts ΔT_1 and ΔT_2 to the temperatures at which the coexistence region is entered and exited would differ. However, as the difference in the partitioning of cholesterol into the two phases would be expected to have the same sign at the two temperatures, and similarly for the difference in the specific entropies and areas, I would expect, from Eq. (13), that the shifts ΔT_1 and ΔT_2 would have the same sign even though their magnitudes would differ. A similar argument can be made for a multicomponent membrane which exhibits a miscibility transition. If the external constraints were such that the temperature changes in the two-phase region as one phase is converted into the other, then one can expect that, upon the addition of a solute, the temperatures at which the two-phase region is entered and exited will be shifted by amounts of the same sign but of different magnitude.

IV. CONCLUSIONS

I conclude with a few observations and predictions. First I noted in the Introduction that the results of Gray *et al.* [4] were interesting, *inter alia*, because the introduction of short-chain alcohols reduced the lo-ld miscibility transition, whereas the addition of cholesterol caused it to increase. That is now readily understood from Eq. (27) and the fact that short-chain alcohols partition preferentially into the ld phase, that with the larger entropy per particle. Thus the signs of $c^I - c^{II}$ and $s^I - s^{II}$ are the same in Eq. (27). In contrast, cholesterol is known [16] to partition preferentially into the lo phase, the phase with the smaller entropy per particle. Thus $c^I - c^{II}$ and $s^I - s^{II}$ have opposite signs.

Second, the analysis presented here and the calculation of the partition coefficients shown in Fig. 1 predicts that if the addition of an alcohol with $n = 14$ has almost no effect on the transition temperature, then the addition of an alcohol with $n = 16$ will increase the transition temperature. This prediction has recently been confirmed [17].

Third, it can also be seen from Fig. 1 that the addition of alcohols with unsaturated bonds will lower the transition temperature more than those with saturated tails, and that for a given n the magnitude of the decrease in transition temperature will increase with the degree of unsaturation.

I emphasize that the above analysis is relevant for first-order transitions of the solvent in which the solute concentrations, c, are sufficiently small that contributions quadratic in c to the solvent chemical potential can be ignored. How small this is can be estimated from the fact that the energy, being a homogeneous function of order unity, must depend upon the number of solute molecules, n_s, according to

$$U = \frac{1}{2}\frac{n_s^2}{n_s + N} J_1 + \frac{n_s N}{n_s + N} J_2, \tag{28}$$

where, as before, N is the number of solvent molecules. The interaction strengths J_1 and J_2 are those between solute molecules themselves, and between solute and solvent molecules, respectively. Differentiating with respect to N we find the contribution to the chemical potential of solvent molecules is $c^2(J_2 - J_1/2)$. Comparing this with the contribution to the solvent chemical potential which is of first order in the solute concentration, $-k_B T c$, Eq. (10), we see that the arguments of this paper require that the solute concentration be less than $c^* \approx k_B T/J$, where J is the order of magnitude of the larger of the two interaction strengths J_1 and J_2. If the concentration of solute is indeed less than c^*, then the analysis of this paper is applicable to first-order transitions, even those which are close to a critical point, as in the experiments of Gray *et al.* [4].

For concentrations larger than c^* it is well known that a solute which acts like an amphiphile, gaining energy by placing itself between the components of the solvent, decreases the miscibility transition temperature, while one that prefers *either* phase of the phase-separated system increases that temperature [18]. These behaviors were manifest in a recent simulation [14]. Combining these results with those for the small concentrations of the dilute-solution regime, one sees that a solute which prefers the lo phase, the one with the smaller entropy per particle, will raise the transition temperature over a wide range of compositions. In contrast a solute which prefers the ld phase, that with the larger entropy per particle, will on first addition decrease the transition temperature, but on further addition will eventually increase it. From this observation there results a fourth prediction: that a short-chain alcohol which, at small concentrations, had been observed to lower the miscibility transition temperature in a giant plasma membrane vesicle will actually raise that temperature if its concentration in the membrane can be increased sufficiently.

Finally, I note that it has recently been observed [19] that short-chain alcohols added in small concentrations to three-component giant unilamellar vesicles raise the lo, ld miscibility transition temperature, in contrast to their behavior when added to the giant plasma membrane vesicles of Gray *et al.* [4]. I would predict that, all other thermodynamic variables being held constant, smaller concentrations of short-chain alcohol than those used would lower the transition temperature in giant unilamellar vesicles. Of course, I am assuming that the reduction in transition temperature resulting from this small concentration would be observable reliably. The difference between the results for the temperature shift in the two types of membranes could, perhaps, be related to the difference in their compositions which affects not only the partitioning of the solute into the coexisting lo and ld phases, but also the entropy per particle of those phases. Both of these factors, the latter particularly, affect the magnitude of the shift in transition temperature, as can be seen from Eq. (24). Thus the temperature shift in giant unilamellar vesicles might be much smaller than in giant plasma vesicles. The difference in entropy per particle is, of course, directly related to the latent heat of the transition, so just how closely the behavior of the two different vesicles correspond to one another could be interrogated by calorometric methods.

ACKNOWLEDGMENTS

I am grateful for the many rewarding discussions with Caitlin Cornell and Sarah Keller, and also thank Sarah Veatch and Mark Uline for useful correspondence.

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