The Effect of Solutes on the Temperature of Miscibility Transitions in Multi-component Membranes

D. W. Allender\textsuperscript{1,2} and M. Schick\textsuperscript{1}
\textsuperscript{1}Department of Physics, University of Washington, Seattle, Washington
\textsuperscript{2}Department of Physics, Kent State University, Kent, Ohio

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Abstract
We address questions posed by experiments which show that most small-chain alcohols reduce the miscibility transition temperature when added to giant plasma membrane vesicles, but increase that temperature when added to giant unilamellar vesicles. In both systems the change in temperature depends non-monotonically on the length of the alcohol chain. To emphasize the roles played by the internal entropies of the components, we model them as linear polymers. We show that, within Flory-Huggins theory, the addition of alcohol causes an increase or decrease of the transition temperature depending upon the competition of two effects. One is the dilution of the solvent interactions caused by the introduction of solute, which tends to lower the temperature. The other is the preference of the solute for one phase or the other, which tends to raise the temperature. The magnitude of this term depends on the entropies of all components. Lastly we provide a reasonable explanation for the behavior of the transition temperature with alcohol chain length observed in experiment.
1 Introduction

A long-accepted means of interrogating the properties of a bilayer membrane is to add to it a molecule whose properties are well understood. In this spirit, a series of experiments were carried out in which short-chain alcohols were introduced into giant plasma membrane vesicles (GPMVs)\(^1\)\(^2\). It was found that the addition of most of the alcohols caused a decrease in the temperature at which the system transitioned from a single uniform phase to two coexisting liquid-ordered (lo) and liquid-disordered (ld) phases. However, when these same alcohols were introduced into giant unilamellar vesicles (GUVs) composed of a ternary mixture of dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC) and cholesterol, the transition temperature usually increased\(^3\)\(^4\). These two quite disparate results did share one feature; the behavior of the transition temperature changed non-monotonically as a function of the length of the \(n\)-alcohol chain. In the GPMVs, addition of \(n\)-alcohols with \(2 \leq n \leq 10\) reduced the transition temperature (1) while hexadecanol increased it (2). In the GUVs, as shown in Fig. 1, alcohols with \(2 \leq n \leq 8\) increased the transition temperature, those with \(10 \leq n \leq 14\) decreased it, and hexadecanol increased it once again. We want to understand the difference in behavior between the two systems and the origin of the non-monotonic behavior of the transition temperature with the chain length of the alcohol.

![Figure 1: Change in transition temperature upon the addition of \(n\)-alcohol to a GUV comprised of mol fractions 35/35/30 DOPC/DPPC/cholesterol. Concentrations of solute are all three times a fiducial given in Ref. 5. Figure from Ref. 4.](image)

The problem of predicting the effects of a solute on a solvent which can
undergo a phase transition is a rather old one. Over fifty years ago, Prigogine and Defay devoted a chapter to it in their volume “Chemical Thermodynamics” \(6\). By examining a regular solution of structureless components they concluded (a) “Hence the introduction of a third component which is equally soluble in the first two components will lower the critical solution temperature...” and (b) “Hence the addition of a third component which is much less soluble in one of the first two components than in the other, will always raise the critical solution temperature...”.

Recently these statements were made more quantitative by the simulation of a simple Ising model \(7\). In it, the “solvent” spins interacted with their nearest neighbors with a strength \(J\), while a small amount of a third, “solute”, spin interacted with its neighbors with strength \(gJ\). It was found that the transition temperature increased if \(|g| > 1\), and decreased if \(|g| < 1\). The lowest transition temperature occurred when the third component interacts with the other two equally, i.e. when \(g = 0\). The conditions which bring about the lowest transition temperature in a membrane are of interest because the transition temperature scales with the effective interaction between lipids. Furthermore many other properties of the membrane scale with this interaction, such as its internal pressure profile. The circumstances which bring about the lowest transition temperature, then, correspond to a membrane in which this interaction scale is reduced by the greatest amount.

Because of the simplicity of the Ising model, the behavior of the transition temperature depends upon a single parameter, \(g\). Because of the symmetry of the Ising model, which corresponds to the equality of the chemical potentials of the two major components at the same temperature, the entropy of the two coexisting phases are equal. The lack of internal entropy of the components, and the equality of the entropy of the coexisting phases are not characteristic of biological lipid components nor of their phases \(8\,9\). That the difference in entropy of the coexisting phases is of importance was pointed out by one of us \(9\) who noted that it is well-known \(10\) that the temperature of a transition in a one-component system is decreased if a solute partitions preferentially into the phase with greater entropy. It was then shown that the tendency remains in a multi-component membrane. However the difference in the behavior of the transition temperature in GUVs and GPMVs was not explained.

It is the purpose of this paper to illuminate the effect of the internal entropies of membrane components and of solute on the behavior of a miscibility transition temperature as solute is added. We do this by considering membrane components \(A\) and \(B\) as polymer chains of polymerization indices \(N_A\) and \(N_B\), and the solute as a polymer chain of index \(N_S\). The critical
temperature is calculated within mean-field theory as a function of the solute volume fraction. For small volume fractions, comparable to those in the experiments noted above, we find that the behavior of the miscibility transition temperature as a function of solute volume fraction results from a competition between two tendencies: the first is simply that the introduction of any solute reduces the number of solvent interactions and therefore lowers the transition temperature. The second tendency is essentially that noted by Prigogine and Defay: if the solute prefers one phase to the other, that will tend to increase the transition temperature. However the magnitude of this increase depends not only upon the difference in the interactions between the solute and the solvent components, but also upon the intrinsic entropies of the membrane components and of the solute. This term, in general, is not minimum when the solute partitions equally into the two phases, but rather when it partitions somewhat preferentially into the phase with the larger entropy. With the aid of our results, we are able to understand the difference in the behavior of the transition temperature in GPMVs and GUVs. Further, by combining them with previous results for the partitioning of \( n \)-alcohols in \( \text{lo} \) and \( \text{ld} \) phases (11), we are able to provide an explanation for the non-monotonic behavior with chain length of the miscibility transition temperature in membranes containing \( n \)-alcohols.

2 Methods

2.1 The Model

We consider an incompressible membrane at temperature \( T \) comprised of \( n_A \) molecules of component \( A \) and \( n_B \) molecules of component \( B \). In order to consider the effects of internal entropy, we treat the components as linear polymers with polymerization indices \( N_A \) and \( N_B \). To this membrane, we add \( n_S \) molecules of a solute, also treated as a linear polymer with polymerization index \( N_S \). Because the system is incompressible, its volume, \( \Omega \), is not a thermodynamically independent variable, but is related to the number of molecules of the components according to

\[
\Omega(n_A, n_B, n_S) = v_0(n_A N_A + n_B N_B + n_S N_S),
\]

where \( v_0 \) is the monomer volume of \( A \), \( B \), or \( S \) chains, volumes which are assumed to be equal.

In mean-field, or Flory-Huggins, approximation (12), the Helmholtz free
energy of the system, $F$, can be written

$$F(T, n_A, n_B, n_S) =$$

$$V_{AB} n_A N_A \frac{n_B N_B v_0}{\Omega} + V_{AS} n_A N_A \frac{n_S N_S v_0}{\Omega} + V_{BS} n_B N_B \frac{n_S N_S v_0}{\Omega}$$

$$+ k_B T \left\{ n_A \ln \left[ \frac{n_A N_A v_0}{\Omega} \right] + n_B \ln \left[ \frac{n_B N_B v_0}{\Omega} \right] + n_S \ln \left[ \frac{n_S N_S v_0}{\Omega} \right] \right\} \tag{2}$$

where $k_B$ is Boltzmann's constant. The energy $V_{AB}$ is related to the interaction energy between pairs of $A$ monomers, $\tilde{V}_{AA}$, pairs of $B$ monomers, $\tilde{V}_{BB}$, and $AB$ pairs, $\tilde{V}_{AB}$, according to

$$V_{AB} = \tilde{V}_{AB} - \frac{1}{2}(\tilde{V}_{AA} + \tilde{V}_{BB}), \tag{3}$$

and similarly for $V_{AS}$ and $V_{BS}$. From the free energy, the chemical potentials of the three components are obtained

$$\mu_\alpha = \frac{\partial F(T, n_A, n_B, n_S)}{\partial n_\alpha}, \quad \alpha = A, B, S. \tag{4}$$

The chemical potentials are functions of temperature and two other intensive quantities. It is convenient to take them to be the volume fractions of the $A$ and $S$ components

$$\Phi_A \equiv \frac{n_A N_A v_0}{\Omega}, \tag{5}$$

$$\Phi_S \equiv \frac{n_S N_S v_0}{\Omega}. \tag{6}$$

The volume fraction of the $B$ component is then $\Phi_B = 1 - \Phi_A - \Phi_S$.

### 2.2 Method of Solution

Given a net repulsive interaction, $V_{AB} > 0$, there will be a transition from one uniform phase to two coexisting phases, $I$ and $II$, below some critical temperature. The equations which determine the volume fractions of the components at coexistence are

$$\mu_A(T, \Phi_A^I, \Phi_S^I) = \mu_A(T, \Phi_A^{II}, \Phi_S^{II}),$$

$$\mu_B(T, \Phi_A^I, \Phi_S^I) = \mu_B(T, \Phi_A^{II}, \Phi_S^{II}),$$

$$\mu_S(T, \Phi_A^I, \Phi_S^I) = \mu_S(T, \Phi_A^{II}, \Phi_S^{II}). \tag{7}$$
These three equations in five unknowns determine the surface of coexistence $T(\Phi^I_A, \Phi^I_S)$, $\Phi^{II}_A(\Phi^I_A, \Phi^I_S)$ and $\Phi^{II}_S(\Phi^I_A, \Phi^I_S)$.

The line of critical compositions, $\Phi_{A,c} = \Phi_{A,c}(\Phi_S)$, is obtained from the non-trivial solution of the two homogeneous equations in two unknowns

\[
\begin{align*}
\Phi^{II}_A(\Phi^I_A, \Phi^I_S) - \Phi^I_A &= 0, \\
\Phi^{II}_S(\Phi^I_A, \Phi^I_S) - \Phi^I_S &= 0.
\end{align*}
\]

From this line of critical compositions, the critical temperature is obtained as a function of solute volume fraction, $T_c(\Phi_S) = T(\Phi_{A,c}(\Phi_S), \Phi_S)$.

To obtain the properties of the critical line, we expand Eqs. (7) in the small parameters $\Phi^{II}_A - \Phi^I_A$ and $\Phi^{II}_S - \Phi^I_S$. If we keep only terms in linear order, the resulting equations are not independent, but yield the two linear homogeneous equations, Eqs. (8) and (9), noted above. Setting the determinant of these equations to zero, we obtain the following expression for the critical temperature in terms of the unknown critical volume fraction, $\Phi_{A,c}$, and the given solute volume fraction $\Phi_S$;

\[
k_B T_c = \frac{V_{AB} \beta + (\beta^2 + 4\alpha\gamma)^{1/2}}{2\alpha},
\]

where

\[
\begin{align*}
\alpha &\equiv \frac{1}{N_A \Phi_{A,c}} \left[ 1 + \frac{N_S \Phi_S}{1 - N_B(1 - \Phi_S)} \right] + \frac{1}{N_B(1 - \Phi_{A,c} - \Phi_S)} \left[ 1 + \frac{N_S \Phi_S}{1 - \frac{N_A(1 - \Phi_S)}{N_A(1 - \Phi_S)}} \right], \\
\beta &\equiv 2 \left[ 1 + \frac{N_S \Phi_S V_{BS}}{N_A \Phi_{A,c} V_{AB}} + \frac{N_S \Phi_S V_{AS}}{N_B(1 - \Phi_{A,c} - \Phi_S) V_{AB}} \right], \\
\gamma &\equiv N_S \Phi_S \left[ 1 - 2 \left( \frac{V_{AB} + V_{BS}}{V_{AB}} \right) - \left( \frac{V_{AS} - V_{BS}}{V_{AB}} \right)^2 \right].
\end{align*}
\]

We also obtain from the non-trivial solution of the homogeneous equations the ratio $(\Phi^{II}_S - \Phi^I_S)/(\Phi^{II}_A - \Phi^I_A) \equiv M$, which is the slope of the tie-line. It is evaluated at the critical point, $M_c$, and is also expressed in terms of the unknown critical volume fraction, $\Phi_{A,c}$, and the given solute volume fraction $\Phi_S$;

\[
M_c = -N_S \Phi_S \frac{k_B T_c - N_B(1 - \Phi_{A,c} - \Phi_S)(V_{AB} + V_{BS} - V_{AS})}{[k_B T_c - 2N_S \Phi_S V_{BS}]N_B(1 - \Phi_{A,c} - \Phi_S) + k_B T_c N_S \Phi_S}.
\]

Finally, if we include in our expansion of Eqs. (7) terms of third order in the small expansion parameters, we recover a third independent equation

\[
0 = \frac{1}{N_A \Phi_{A,c}^2} - \frac{(1 + M_c)^3}{N_B(1 - \Phi_{A,c} - \Phi_S)^2} + \frac{M_c^3}{N_S \Phi_S^3}.
\]
This, with the other two equations above, determines $\Phi_{A,c}(\Phi_s)$ and $M_c(\Phi_S)$, and $T_c(\Phi_s)$.

3 Results

We first consider the critical concentration, $\Phi_{A,c}(0)$, and critical temperature, $T_c(0)$, in the limit of no solute, $\Phi_S \to 0$. Following the above procedure, we obtain for the critical concentration of the $A$ component the result:

$$\Phi_{A,c}(0) = \sqrt{N_B} \sqrt{N_A + N_B},$$  \hspace{1cm} (13)

and find the critical temperature to be given by

$$2 \frac{V_{AB}}{k_B T_c(0)} = \left[ \frac{1}{\sqrt{N_A}} + \frac{1}{\sqrt{N_B}} \right]^2.$$  \hspace{1cm} (14)

In the symmetric case for which $N_A = N_B \equiv N$, these results reduce to $\Phi_A = \Phi_B = 1/2$ and $NV_{AB}/k_B T = 2$. (In the polymer literature, $V_{AB}/k_B T$ is denoted $\chi$ so that this relation is written $\chi_c N = 2$, a well-known result.)

Now let the solute be introduced. With the interactions between solute and the solvent components non-zero, we find it convenient to characterize them by the average interaction, normalized by $V_{AB}$ and the difference in the interactions, again normalized;

$$r \equiv \frac{V_{AS} + V_{BS}}{2V_{AB}},$$

$$\delta r \equiv \frac{V_{AS} - V_{BS}}{V_{AB}}.$$  \hspace{1cm} (15)

We now solve the equations for the critical temperature in a power series in the solute volume fraction. Introducing

$$\epsilon \equiv \sqrt{N_A + N_B},$$

$$\nu \equiv \sqrt{N_A} - \sqrt{N_B},$$  \hspace{1cm} (16)

we obtain

$$M_c = -\frac{N_S}{2N_A N_B} \epsilon (\nu + \epsilon \delta r) \Phi_S + O(\Phi_S^2),$$  \hspace{1cm} (17)

$$\frac{\Phi_{A,c}(\Phi_S) - \Phi_{A,c}(0)}{\Phi_{A,c}(0)} = -\left[ 1 - N_s (\nu + \epsilon \delta r) \left( 3 - (\nu + \epsilon \delta r)^2 \frac{N_S}{4N_A N_B} \right) \frac{N_A^{1/2}}{4N_A N_B} \right] \Phi_s + O(\Phi_S^2),$$  \hspace{1cm} (18)
\[
\frac{T_c(\Phi_S) - T_c(0)}{T_c(0)} = c_1\Phi_S + c_2\Phi_S^2 + O(\Phi_S^3),
\]

\[
c_1 = -1 + \frac{N_S}{4N_AN_B}(\nu + \epsilon\delta r)^2,
\]

\[
c_2 = -\left[\frac{N_S}{4N_AN_B}(\nu + \epsilon\delta r)^2\right]\{\epsilon^2(1 - 4r + (\delta r)^2)
\]

\[+ (N_AN_B)^{1/2}(c_1)^2}. \quad (19)
\]

Before discussing the effect of the solute on the transition temperature, we simply note the following: if \(\delta r > 0\), the solute prefers the B-rich phase, hence a negative slope of the tie-line, as defined above, is expected at the critical point. That this is the case is shown by Eq. (17). The change in the volume fraction of component A at criticality is a result of two effects. The first term in Eq. (18) is simply the effect of dilution; that is, as a volume fraction of solute is introduced, the volume fraction of the other components must decrease. The second term shows that if the solute prefers the B-rich phase, this will tend to increase the volume fraction of the A component at the critical point. Note that the presence of the factor \(\nu\) expresses a purely entropic effect.

For the shift in the critical temperature due to the addition of solute, which is the principal result of this paper, we note that for the millimolar concentrations employed in the experiments of interest, it is sufficient to keep in Eq. (19) only the term linear in \(\Phi_S\),

\[
\frac{T_c(\Phi_S) - T_c(0)}{T_c(0)} = \left[-1 + \frac{N_S}{4N_AN_B}(\nu + \epsilon\delta r)^2\right]\Phi_S. \quad (20)
\]

From this expression, one sees that the effect on the temperature at criticality, just as on the concentration of component A at criticality, results from a competition between two terms. The first, \(-\Phi_S\), is simply the reduction in the transition temperature due to the dilution of the number of AB interactions resulting from the introduction of the solute. This effect would be present no matter the nature of the solute. The second term, being positive, always tends to increase the transition temperature. It reflects, but modifies, the dictum of Prigogine and Defay (6) that "...the addition of a third component which is much less soluble in one of the first two components than in the other, will always raise the critical solution temperature...". Indeed if \(\delta r = (V_{AS} - V_{BS})/V_{AB}\) is large in magnitude, and of either sign, then this term will tend to increase the transition temperature. Whether it will actually do so or not depends upon the intrinsic entropies of the solute as well
as those of the solvent, $A$ and $B$. It is interesting that this effect depends only on the magnitude of the difference, $\delta r$, between the solute interactions; it is independent of the strength of these interactions, encapsulated in the parameter $r$. Furthermore we see that the largest reduction of the transition temperature does not occur when the solute interacts equally with the solvent components $A$ and $B$, i.e. $\delta r = 0$, but rather when there is a difference in its interactions equal to

$$
\delta r = \frac{V_{AS} - V_{BS}}{V_{AB}}
$$

Recalling that a positive interaction is repulsive, we see that if $N_A > N_B$, then the above states that the greatest reduction in transition temperature occurs when the solute partitions preferentially into the $A$-rich phase. As we show below, near the critical transition, this phase is the one with larger entropy per unit volume. Hence this nicely illustrates the point made in Ref. (9) that if the solute partitions preferentially into the phase with greater entropy, the transition temperature of the multi-component system will tend to decrease. Again, whether it actually does so depends on the magnitude of this term compared to unity.

To see that the entropy per unit volume of the $A$-rich phase, phase $I$, is greater than that of the $B$-rich phase, phase $II$, near the critical point, if $N_A > N_B$, we note that, from Eqs. (2) and (5), in the absence of solute, the entropy per unit volume, $S/\Omega$, is given by

$$
\frac{Sv_0}{\Omega k_B} = - \left[ \frac{\phi_A}{N_A} \ln(\phi_A) + \frac{(1 - \phi_A)}{N_B} \ln(1 - \phi_A) \right].
$$

The volume fraction $\Phi_A$ in the $A$-rich phase can be written $\Phi_A = \Phi_{A,c} + \delta\Phi_A$ with $\delta\Phi_A > 0$. Near the critical point, $\delta\Phi_A/\Phi_{A,c}$ is small, so the above can be expanded in this parameter with the result

$$
\frac{(S_I - S_{II})v_0}{\Omega k_B} \approx -2 \left\{ \left[ \frac{\ln(\phi_{A,c}) + 1}{N_A} - \frac{\ln(1 - \phi_{A,c}) + 1}{N_B} \right] \right\} \delta\Phi_A.
$$

Finally setting $N_A = N + \delta N$, $N_B = N - \delta N$ with $\delta N > 0$ and expanding in $\delta N/N$, we obtain

$$
\frac{(S_I - S_{II})v_0}{\Omega k_B} \approx 2(3 - 2 \ln 2) \frac{\delta N}{N^2} \delta\Phi_A \approx 3 \frac{\delta N}{N^2} \delta\Phi_A
$$

which is positive.
4 Discussion

As we have seen, the resolution of the question of whether the addition of a solute to a solvent that can undergo a miscibility phase transition will raise or lower the transition temperature depends on the competition between two effects. The first, which is independent of the nature of the solute, is simply that the introduction of a solute reduces the number of interactions between solvent components and therefore tends to lower the transition temperature. The second depends on the nature of the solute. If it strongly favors one phase or the other, its introduction tends to raise the transition temperature. The magnitude of this second effect, and therefore the result of the competition between terms, depends upon the internal entropies of solvent and solute components. The behavior of the transition temperature is neatly encapsulated in the behavior of the quantity $c_1$ in Eqs. (19) and (20),

$$c_1 = -1 + \frac{N_S}{4N_AN_B} (\nu + \epsilon \delta r)^2.$$  

(25)

If it is positive, the transition temperature increases, if negative the transition temperature decreases. We can understand from this how it can come about that the addition of short-chain alcohols to GPMVs causes a decrease in the transition temperature while the same alcohols in GUVs can cause an increase. First, the GPMVs contain long chain poly-unsaturated fatty acids which the GUVs do not. As a consequence the effective chain length, $N_A$, of the component which is more abundant in the liquid-disordered phase is larger in GPMVs than it is in GUVs. This decreases the prefactor of the second term in the expression for the shift in transition temperature, Eq. (20), thereby diminishing the effect of the interactions and making a decrease in the transition temperature more likely. This is an entropic effect. Second, it can be argued (4) that the difference in the order between the lo and ld phases is less in GPMVs than in GUVs, hence the difference in interactions, $\delta r$, between solute and solvent components is less. This also makes a reduction in the transition temperature more likely.

To understand the variation in $T_c(\Phi_S)$ with the length of the n-alcohol chain, we must understand the behavior of the partitioning of the alcohol into the two phases, lo and ld, as a function of $n$. Fortunately the partition coefficient, the ratio of the fraction of alcohol in the lo phase to that in the ld phase, has been calculated for a series of acyl chains in a membrane composed of DPPC, DOPC, and cholesterol (13). The results for saturated chains and various unsaturated chains are shown in Fig. 2.

From the figure, we see that short-chain n-alcohols with $n < 14$ par-
Figure 2: 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, while $C_n: 1 c9$ denotes a chain of length $n$ with one double bond at the ninth position, etc. From Ref. [13].

Partition preferentially into the ld phase, from which we infer $\delta r$ is negative. Presumably this preference is due to the area per lipid head group being larger in that phase. For these values of $n$, we do not expect the magnitude of $\delta r$ to be small. With increasing $n$, the energy penalty of repulsive interactions between the alcohol chain and the DOPC chain due to its double bond increases. In addition the favorable energy of interaction with the ordered chains of the DPPC increases. Insertion of the alcohol into the lo phase is opposed, however, by the cholesterol. As a consequence of these various factors, there will be a range of $n$ for which the alcohol partitions roughly equally into the two phases. In this range $\delta r$ is small. Eventually for sufficiently large $n$, on the order of 16 to 18, the n-alcohol partitions predominantly into the lo phase. For such values of $n$, $\delta r$ is once again not small, but now is positive. Based on these observations and our analysis encapsulated in Eq. [25] we can predict that, (i), even in the GUVs, for which most alcohols increase the transition temperature, there will be an interval of $n$ over which the transition temperature decreases on the addition of n-alcohols. That this is indeed the case is seen in Fig. 1. Furthermore because of the presence of the entropic term $\nu = \sqrt{N_A} - \sqrt{N_B} > 0$ in Eq. [25], we would predict, (ii), that the onset of the interval in $n$ over which the transition temperature decreases in the GUVs will occur when the alcohol still partitions more favorably into the ld phase. That this is correct is seen by a comparison of Figs. 1 and 2.

There are other predictions that can be made on the basis of Eq. [25].
For example, we expect that the change in transition temperature would be larger in membranes composed of shorter lipids than in those of longer ones, and that the effect will be larger were chains with double bonds to be added instead of the saturated alcohols. Of course any molecule that strongly prefers one phase to the other will tend to increase the transition temperature. There are many proteins that would be expected to do this, and the effect should be observable upon the introduction of simple peptides.

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