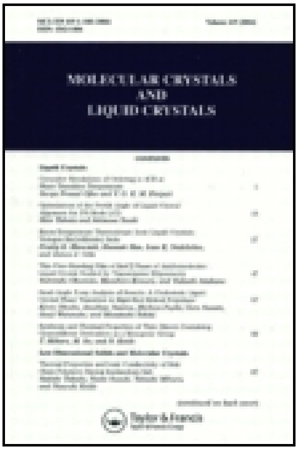

Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

Landau Theory of One and Two-Component Phospholipid Bilayers: II. Excess Heat Capacity Functions and Cooperative Unit Sizes

IstvÁN P. Sugár [a] [c] & Gianluigi Monticelli [b]

[a] Semmelweis Medical University, Institute of Biophysics, Budapest, 1444, POB 263, Hungary

[b] Dipartimento di Fisiologia e Biochimica generali dell'Universita degli Studi, Via Celoria 26, 20133, Milano, Italy

[c] Department of Biochemistry, University of Virginia, Box 440, Charlottesville, Virginia, 22908

To cite this article: IstvÁN P. Sugár & Gianluigi Monticelli (1984): Landau Theory of One and Two-Component Phospholipid Bilayers: II. Excess Heat Capacity Functions and Cooperative Unit Sizes, Molecular Crystals and Liquid Crystals, 112:3-4, 197-212

To link to this article: http://dx.doi.org/10.1080/00268948408071835

Mol. Cryst. Liq. Cryst., 1984, Vol. 112, pp. 197–212
0026-8941/84/1124-0197/$20.00/0

Landau Theory of One and Two-Component Phospholipid Bilayers: II. Excess Heat Capacity Functions and Cooperative Unit Sizes

ISTVÁN P. SUGÁR†
Semmelweis Medical University, Institute of Biophysics
Budapest 1444 POB 263 Hungary

and

GIANLUIGI MONTICELLI
Dipartimento di Fisiologia e Biochimica generali dell'Universitá degli Studi,
Via Celoria 26, 20133 Milano, Italy

(*Received December 14, 1983; in final form May 23, 1984*)

Improving the Priest model[6] not only the phase transition temperature and the phase diagrams of one- and two-component lecithin homologues‡ but also the excess heat capacity curves and cooperative unit sizes are determined. Here we used a new method to determine the cooperative unit size. It is pointed out that this method and the generally used expression, the ratio of van't Hoff enthalpy to the calorimetric enthalpy ($\Delta H_{vH}/\Delta H_{cal}$) result in the same values for the cooperative unit size if the partition function can be approximated by means of the maximum-term method. This condition does not fulfil for one-component lecithin membranes, consequently the cooperative unit sizes obtained by the two methods differ by a 1.5–2 factor. A great advantage of this new method that one can determine the cooperative unit size of multicomponent

---

† To whom correspondence should be addressed. Dr. Sugar, Department of Biochemistry, University of Virginia, Box 440, Charlottesville, Virginia 22908

‡ Abbreviations: DLPC, *L*-α-dilauroylphosphatidylcholine; DMPC, *L*-α-dimyristoylphosphatidylcholine; DPPC, *L*-α-dipalmitoylphosphatidylcholine; DSPC, *L*-α-distearoylphosphatidylcholine.

systems too. As regards to the two-component systems, we can state the following:

1. The shapes of the excess heat capacity curves depend strongly on the values of the cooperative unit sizes.
2. By means of the model, the experimentally obtained excess heat capacity curves can be reproduced.
3. The mixing of the components results in the drastic decrease of the cooperative unit size.
4. During mixing, the greater the chain length difference between the components is, the stronger the decrease of the cooperative unit size.

INTRODUCTION

A number of theoretical works have been performed to interpret the phase diagrams of two-component lecithin bilayers, namely, statistical mechanical[1-5] and phenomenological models.[6-7]

In this paper Priest's phenomenological model,[6] generalized previously for phosphatidylcholine/phosphatidylethanolamine mixtures,[9] has been further developed. In addition to the phase diagrams, we determine the excess heat capacity curves of one and two-component lecithin membranes as well. By means of these calculations, trying to fit experimental data,[10] the cooperative unit sizes of the mixed systems are also determined.

SMALLEST SUBSYSTEM AND COOPERATIVE UNIT

At the critical point the correlation length of the system, going through a second order phase transition, is infinite.[16] During a first order phase transition (e.g. gel–liquid crystalline phase transition of the lipid membranes) the correlation length of the system remains finite, and the system can be separated into mathematically independent subsystems. These subsystems are characterized with the same parameters. For instance the ratio of the gel/liquid crystalline phase agrees in the subsystems and in the multicomponent cases the ratios of the different components agree in the subsystems too. The linear size of these independent subsystems higher or comparable with the correlation length. As a consequence of the independence the phase transition properties of a subsystem with continuous functions across the boundary (e.g. transition temperature, transition enthalpy/mole etc.) agree with that of the total system. The maximum of the correlation length belonging to the examined phase transition determines the minimum size of the independent subsystems. In general, subsystems being smaller than this minimum size cannot be considered to be independent from each other. Consequently the partition function of the total system (Q) can be factorized into the partition functions of the smallest subsystems (Q_{sub}):

$$Q = Q_{\mathrm{sub}}^{M/\mathcal{N}} = \left[\int_S \exp - \{ f(S,T) \cdot \mathcal{N} / kT \} \, dS \right]^{M/\mathcal{N}}$$

where M and $\mathcal{N}$ are the numbers of interacting units (molecules) in the total system and that of in the smallest independent subsystem respectively. $f(S, T)$ is the specific free energy function, S is the order parameter characterizing the phase transition and T is the absolute temperature.

It is pointed out in the Appendix that the size of the smallest subsystem is equal to the cooperative unit size if the partition function can be approximated by its maximum term. Namely one can derive the well known formula of the cooperative unit size ($\mathcal{N}_0$):[8,11–13]

$$\mathcal{N}_0 = \Delta H_{\mathrm{vH}} / \Delta H_{\mathrm{cal}} = 4RT_{\mathrm{m}}^2 \left. \frac{d\theta}{dT} \right|_{T_{\mathrm{m}}} \cdot \Delta H_{\mathrm{cal}}^{-1} \qquad (1)$$

for the size of the smallest subsystem too. In Eqn. 1 ΔH_{vH}, ΔH_{cal}, T_{m} and θ are the van't Hoff enthalpy, calorimetric enthalpy change, midpoint temperature and degree of phase transition respectively. However it is important to note that in spite of this equivalence on phenomenological level the definitions of these concepts are different on molecular level! According to Stankowski and Gruenewald:[8] ‘ ‘ . . . the cooperative unit represents an average cluster of strongly correlated molecules at the midpoint of the transition . . . ”, where a cluster contains molecules being in only one of the coexisting phases.

In the next sections we determine the size of the smallest subsystems for one and two-component lecithin membranes. Since this size is equivalent with the size of the cooperative unit it is also called cooperative unit size in this study. The main advantage of this method is that one can easily determine the cooperative unit size of the multicomponent systems too.

MODEL OF ONE-COMPONENT BILAYERS

Applying the Landau theory of phase transitions, Priest[6] constructed probably the simplest model of phospholipid bilayers. According to this model, the free energy per mole of hydrocarbon chains (one chain consists of L CH_2 units) is given by:

$$\begin{aligned} F/2RT &= E_0(L-3)S/RT + (L-L^*) \\ &\qquad \times\left[W(S-S_0)^2 - Z(S-S_0)^3 - W(S_0)^2 - Z(S_0)^3\right]/RT \\ &\qquad - (L-2)2\sqrt{2}\,(S-S^2) \qquad \text{at} \quad S \geqslant 0, \\ F/2RT &= \infty \qquad\qquad\qquad\qquad\qquad\quad \text{at} \quad S < 0, \end{aligned} \tag{2}$$

where S is the order parameter (the fraction of C-C bonds in the hydrocarbon chain tails which are in gauche conformation), E (= 500 cal/mol $\cdot$ CH_2 = 2.1 kJ/mol $\cdot$ CH_2) is the energy associated with the trans-gauche energy difference, R is the universal gas constant, T is the absolute temperature. The fitted model parameters L^*, W/R and Z/R are 6.4, -1085 K and -275.1 K respectively; $S_0 = 0.39$ is the value predicted for S at the melting point of Extended Chain Conformation (ECC) polyethylene.[6] The first term of Eqn. 2 is associated with the intramolecular conformation of molecules. The second contribution is due to the change in the optimal value of the density connected with the changes in S, i.e. to the change of intermolecular conformation. The third one is the entropy term.

The changes in excess enthalpy or excess heat capacity characterize changes in structural state of the membrane. In the case of condensed systems the mechanical working term of Gibbs free energy function is negligible[17] and the specific enthalpy function of the system can be calculated by means of the following formula:

$$H(S) = -T^2\partial(F/T)/\partial T = F(S) + 2\sqrt{2}\,(L-2)(S-S^2)2RT \tag{3}$$

In order to obtain the average specific enthalpy ($\bar{H}$), over the order parameter, we have to take the following into consideration:

i. the order parameter can change in the $0 \leqslant S \leqslant 0.5$ interval,[6]

ii. the intermolecular interaction term in Eqn. 2 is constant if $S > S_0$ and its value is:[6]

$$(L-L^*)(-WS_0^2 - ZS_0^3)/RT,$$

iii. the average specific free energy of the smallest subsystem equals that of the total system and can be calculated by the following formula:[14]

$$\bar{F} = -(RT/\mathcal{N})\ln Q_{\text{sub}}$$

Taking these notes and Eqn. 3 (for averages) into consideration the average specific enthalpy ($\overline{H}$) is as follows:

$$\overline{H(T)}/2R = \int_0^{1/2}[H(S)/2R] \cdot \exp[-F(S)N/2RT]\,dS \Big/ \int_0^{1/2}\exp[-F(S)N/2RT]\,dS \tag{4}$$

where N is the number of cooperating hydrocarbon chains and $\mathcal{N} = N/2$ the cooperative unit size.

Substituting Eqns. 2, 3 into Eqn. 4 by means of a numerical integration the average enthalpy in the function of temperature can be calculated. In Fig. 1 these calculated average specific enthalpy curves are shown in case of DPPC membrane ($L = 16$) at different cooperative unit sizes. Obviously, the steepness of the gel → liquid crystalline phase transition increases with the increasing cooperative unit size. At a suitable value of $\mathcal{N}$, one can reproduce theoretically the experimentally obtained slopes of the enthalpy curves at phase transition temperature.

For the fitting procedure similarly to Priest[6] we use the experimental data of Mabrey and Sturtevant.[10] Though in the Table I of Ref. 10

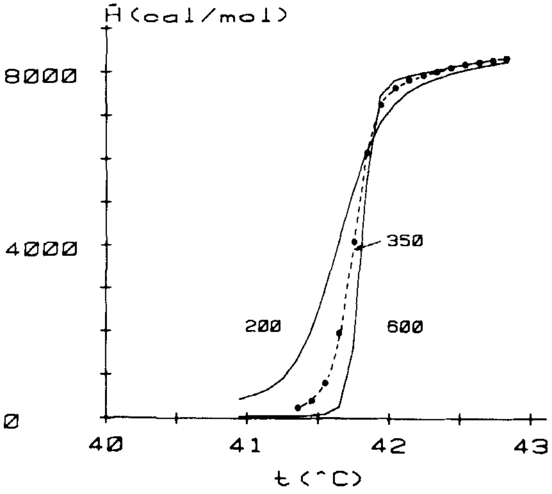

FIGURE 1 Calculated average specific enthalpy of one-component DPPC bilayer in the function of temperature at different values of cooperative unit sizes (solid lines). Experimental data:[12] dotted line.

the authors do not give information on the steepness of the phase transition, these values can, however, be determined unambiguously since Mabrey and Sturtevant used the well-known formula (Eqn. 1) to obtain the cooperative unit size, $\mathcal{N}_0$, where $(\delta\theta/dT)_{T_m}$ is the steepness of the phase transition at $\theta = 1/2$. Thus, as an example, the steepness of the phase transition of DPPC dispersion is:

$$(d\theta/dT)_{T_m} = 2.858\ \mathrm{K}^{-1}$$

using the data reported by Mabrey and Sturtevant,[10] namely, $\mathcal{N}_0 = 260$, $\Delta H_{\mathrm{cal}} = 8700$ cal/mol, $T_m = 314.56$ K. The same steepness was obtained for the enthalpy curve of DPPC at $\mathcal{N} = 350$ from our model. The results of these fitting procedures are shown in Table I for lecithin homologues.

It is seen that the cooperative unit sizes, $\mathcal{N}$, determined by our model are higher by a 1.5–2 factor than the $\mathcal{N}_0$ values, since the maximum term approximation is not applicable for the partition function of the lipid membranes. Namely, there are large fluctuations near the phase transition[16] of one-component lipid membranes and consequently the barrier height between the local minima of the free energy function of the subsystem, $\mathcal{N} \cdot f(S, T)$ is in the order of kT (thermal energy unit). Just in the case of so shallow minima the maximum term approximation is not applicable.

MODEL OF TWO-COMPONENT BILAYERS

The above-described method for the determination of enthalpy curves and cooperative unit sizes can be applied in the cases of two-component bilayers as well. The importance of this method is especially striking here, since the application of Eqn. 1. is impossible even

TABLE I

Some transition properties of lecithin series homologues.[10] Cooperative unit sizes were determined according to Eqn. 1 ($\mathcal{N}_0$) and by means of the proposed model ($\mathcal{N}$)

L	$\mathcal{N}$	ΔHcal (kcal/mol)	$\mathcal{N}_0$	T_m (°C)	$(d\theta/dT)_{T_m}$ (K^{-1})
12	2500	1.7	980	− 1.8	2.83
14	500	5.4	330	23.9	2.52
16	350	8.7	260	41.4	2.86
18	150	10.6	130	54.9	1.60

formally in case of two-component systems, namely, neither the phase transition temperature nor the steepness of the phase transition can be determined.

Applying Priest's result[6] we can write for the state function of a two component bilayer (mixture of phospholipids differing only in chain length):

$$\frac{G(S,X)}{2RT} = \frac{\tilde{F}(S)}{2RT} + \frac{1}{2}X\ln X + \frac{1}{2}(1-X)\cdot\ln(1-X)$$

$$+\Delta U\cdot X + \Delta L\cdot\frac{\sigma}{RT}\cdot\left(\frac{1}{2}-S\right)\cdot X(1-X). \qquad (5)$$

Here $\tilde{F}(S)$ is given by Eqn. 2 with $L = L_s + X\Delta L$, where L_s is the number of CH_2 units of the shorter chain species, ΔL is the chain length mismatch and X is the mole fraction of longer chains. The second and third terms in Eqn. 5 are the entropy of mixing. The factor $\frac{1}{2}$ comes from the fact that there are two chains per head group. The difference in chemical potential between the long and short chains is denoted by ΔU (in dimensionless unit). It is an intensive thermodynamic variable conjugated to X. By adjustment of ΔU, the minimum of $G(S,X)$, with respect to X, can be made to fall at the value of X set in an experiment.

The last term in Eqn. 5 represents the non-ideal effects. It vanishes in a maximally disordered system ($S = \frac{1}{2}$) and has a maximum for the gel state ($S = 0$). The form $X(1-X)$ is the simplest expression that vanishes in both pure component limits. σ/R, is a model parameter. For lecithin mixtures it is 125 K (see Ref. 6). If $\Delta L = 2$ and 4, there are two local minima of $G(X,S)$. One is at $S = 0$ and a larger value of X. This is the long chain rich gel minimum. The other minimum is at a liquid crystalline value of S and a small value of X. This is the short-chain rich liquid crystalline minimum. As a function of ΔU and T, the equilibrium values of X and S correspond to the global minimum of $G(S,X)$. At a given T, one can find a critical value of $\Delta U(=\Delta U_c)$ where both the local minima are global minima of $G(S,X)$.

On the phase diagrams, the pairs of X values (X_g, X_{lc}) belonging to these double global minima are plotted against the temperature (see Fig. 2).

The upper curves of phase diagrams are the so-called liquidus, and the lower ones, the solidus curves. The liquid crystalline phase is above the liquidus curve, and the gel phase is below the solidus curve. Between these curves there is a mixed phase region where short-chain

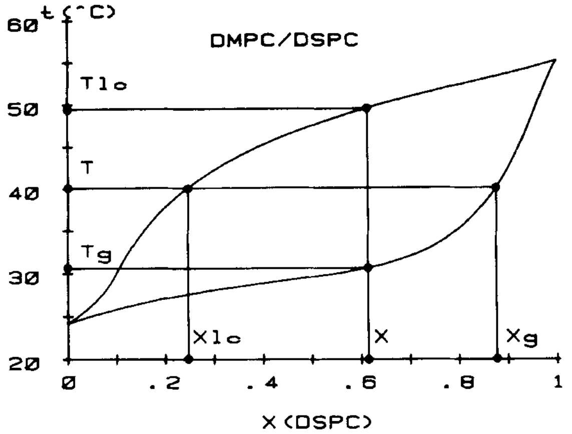

FIGURE 2 Calculated phase diagram of DMPC/DSPC mixture. The indicated characteristic values of the diagram occur in the formula of the average enthalpy, $\overline{H(X,T)}$ (see Eqns. 6, 8).

rich liquid crystalline phase coexists in equilibrium with a long-chain rich gel phase. In the range of *pure gel or pure liquid crystalline phase*, the average specific enthalpy, at a given X and T ($< T_g(X)$ or $> T_{lc}(X)$ see Fig. 2), can be determined easily by the expression analogous to Eqn. 4:

$$\overline{H(X,T)}/2R$$

$$= \int_0^{1/2} \left[H(X,S)/2R \right] \frac{\exp\left[-G(X,S)N/2RT \right] dS}{\int_0^{1/2} \exp\left[-G(X,S)N/2RT \right] dS} \tag{6}$$

where the specific enthalpy function of the mixture, $H(X,S)$ is:

$$H(X,S) = X \cdot H_l(S) + (1-X) \cdot H_s(S)$$

$$+ 2 \cdot \Delta L \cdot \sigma \cdot (0.5 - S)X(1-X) \tag{7}$$

containing the specific enthalpy functions of the pure longer chain system (H_l) and that of the shorter chain system (H_s) (see Eqn. 3) and the third term is the excess enthalpy function. In the mixed phase range, one can obtain the average specific enthalpy at a given X and

$T(T_g(X)T_g(X) < T < T_{lc}(X)T_{lc}(X))$ by means of the lever rule:[14]

$$\overline{H(X,T)}/2R = \frac{X_g - X}{X_g - X_{lc}} \cdot \frac{\overline{H(X_{lc},T)}}{2R} + \frac{X - X_{lc}}{X_g - X_{lc}} \cdot \frac{\overline{H(X_g,T)}}{2R}, \tag{8}$$

where X_g and X_{lc} are the mixing rates belonging to the solidus and liquidus curves, respectively, at a temperature T (see Fig. 2). $\overline{H(X_g,T)}$ and $\overline{H(X_{lc},T)}$ are the average specific enthalpies which can be determined by Eqns. 6–7. We note here that the lever rule is but a two-state approximation, resulting in an error in the calculated excess heat capacity curves.

RESULTS AND DISCUSSION

Cooperative unit size and the shape of the excess heat capacity curves

In Fig. 3 the calculated excess heat capacity curves of DMPC/DPPC mixture are shown at a constant mixing rate ($X = 0.372$), but at

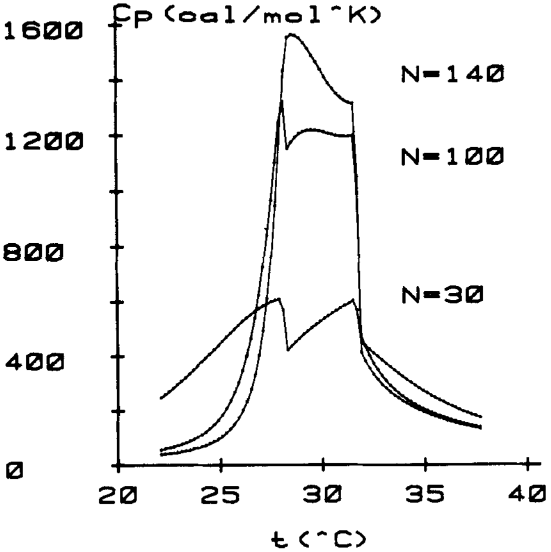

FIGURE 3 Calculated excess heat capacity of two-component DMPC/DPPC mixture in the function of temperature at different values of cooperative unit sizes $\mathcal{N}$ ($= N/2$). Mixing rate is $X = 0.372$.

different cooperative unit sizes, $\mathcal{N}$ ($= N/2$). According to this result, the cooperative unit size has a strong influence on the shape of the heat capacity curves. The broadening and the decrease of the peaks are the main tendencies with decreasing cooperative unit size. This result is in accordance with the experimental findings; i.e. with the increasing amount of impurities, the broadening and the decrease of the calorimetrical peaks were observed.[15]

Cooperative unit sizes of two component-bilayers

The sensitivity of the shape of heat capacity curves to the value of the cooperative unit size ensures its determination with high accuracy during the fitting procedure.

In Fig. 4, the experimental heat capacity curves and the respective theoretical curves are shown for two DMPC/DPPC mixtures. According to this figure, the best fit was obtained at $\mathcal{N} = 70$ ($X = 0.372$) and $\mathcal{N} = 40$ ($X = 0.644$). Nevertheless, the peaks are broader than the experimental ones. These discrepancies may originate from the inaccurate determination of the onset and the end point of the phase transition from the heat capacity curve (Mabrey and Sturtevant[10]) which leads to the inaccuracy of the phase diagram by which the beginning and the end points of the mixed phase were given in our model. In Fig. 5 and 6, the calculated and measured heat capacity curves of DMPC/DSPC and DLPC/DSPC mixtures are shown, respectively.

It is necessary to mention here that DLPC/DSPC mixture determines a so-called peritectic system which differs qualitatively from the earlier ones. (see Fig. 6 in Ref. 6). The phase diagram of this system contains a horizontal line at about 1°C, separating two types of mixed phases. Below 1°C, a short chain rich gel and a long-chain rich gel phase are mixed, while, above it, a short-chain rich liquid crystalline phase and long-chain rich gel phase mix. At a given X, going through this horizontal phase line, the system goes from a complete gel state ($S = 0$) into a partly liquid crystalline state ($S \neq 0$); i.e. the average enthalpy of the system changes discontinuously, and the heat capacity curve has to be a Dirac delta function at this point (see Fig. 6). Therefore, the relative heights of the sharp peaks depend on the enthalpy change at the discontinuity.

On the abscissas of Figs. 4–6, the onset and the end point of the mixed phase region are marked by arrows showing up and down respectively. In the frame of the model, these points are determined with precisity, and their place relative to the characteristic parts of the heat capacity curves can give some ideas for practical cases as well, to

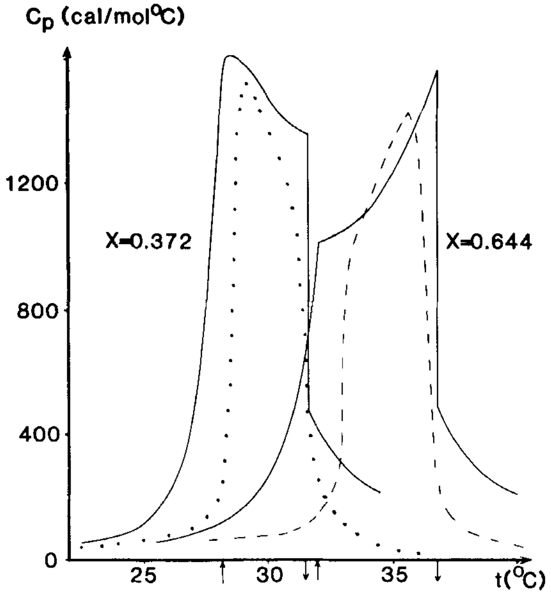

FIGURE 4 Excess heat capacity, C_p of two-component DMPC/DPPC mixture in the function of temperature, at two mixing rates ($X_a = 0.372$ and $X_b = 0.644$). Calculated curves with $\Gamma_a = 70$, $\Gamma_b = 40$, (solid line) and experimental curves[10] (dotted and dashed line).

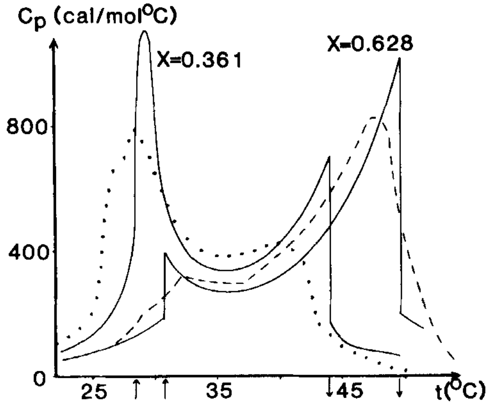

FIGURE 5 Excess heat capacity, C_p of two-component DMPC/DSPC mixture in the function of temperature, at two mixing rates ($X_a = 0.361$ and $X_b = 0.628$). Calculated curves with $\Gamma_a = 20$, $\Gamma_b = 10$, (solid line) experimental curves[10] (dotted and dashed line).

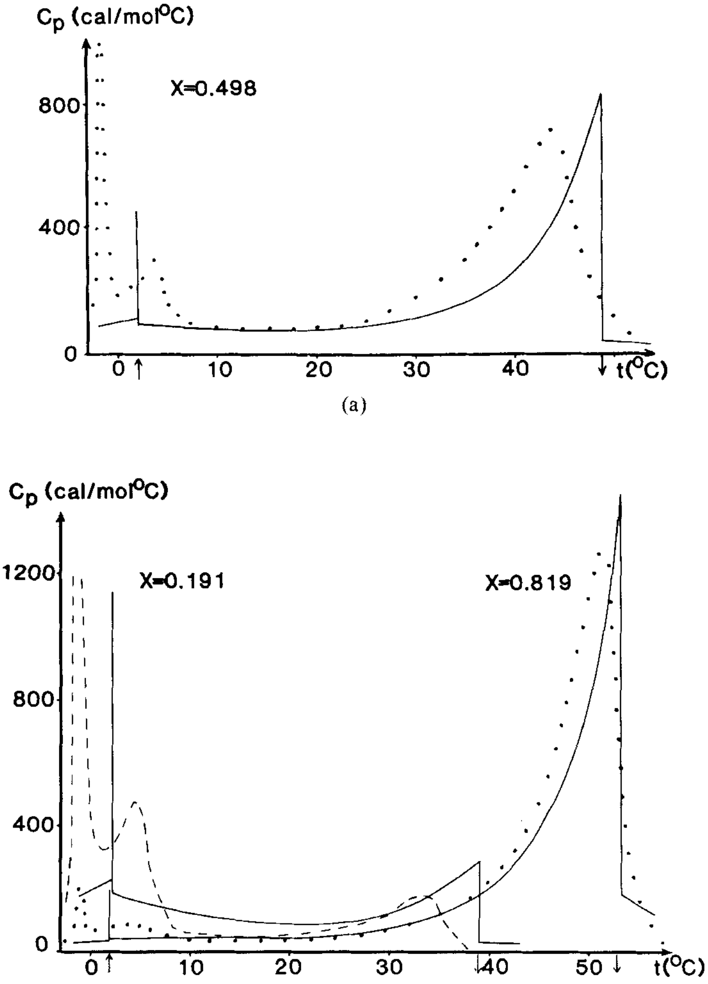

FIGURE 6 Excess heat capacity, C_p of two-component DLPC/DSPC mixture in the function of temperature, at three mixing rates. The theoretical curves (solid) were calculated with . $/_a = 7.5$ at $X_a = 0.498$ (fig. a), . $/_a = 10$ and . $/_b = 7.5$ at $X_a = 0.191$ and $X_b = 0.819$ (fig. b). Experimental curves:[10] dashed and dotted line.

have an approximation for the beginning and the end of the mixed phase region.

In Table II, the fitted values of the cooperative unit sizes are shown in case of different binary mixtures and mixing rates.

TABLE II

Cooperative unit size values determined by fitting procedure of experimental excess heat capacity curves

Mixtures (A/B)	X_B	$\mathcal{N}$
DMPC/DPPC	0	500
	.372	70
	.644	40
	1	350
DMPC/DSPC	0	500
	.361	20
	.628	10
	1	150
DLPC/DSPC	0	2500
	.191	10
	.498	7.5
	.819	7.5
	1	150

According to Figs. 4–6, it can be stated that the model can reproduce not only the phase diagrams but the basic characteristics of the experimentally obtained excess heat capacity curves of two-component lipid bilayers. We could determine the value of cooperative unit size of the two-component systems in every case, and these values depend strongly on the mixing rate and on the types of the mixed molecules. The main tendencies are the following:

i. Mixing always leads to a drastic decrease in the cooperative unit size.

ii. The greater ΔL (chain length difference between the mixed components) is, the stronger decrease of the cooperative unit size during the mixing can be.

iii. The lowest limit of the cooperative unit size in the binary mixtures of the phospholipids is about seven.

These qualitative behaviors of the cooperative unit sizes in the mixed systems will be explained by a simple statistical mechanical model in the subsequent paper. Here a phenomenological determination of the cooperative unit size was given. While $(d\theta/dT)|_{T_m}$, T_m and ΔH_{cal} can be determined directly from the calorimetrical spectra in certain cases, the parameters of the Priest model are obtained after a fitting procedure. Obviously both sets of parameters depend implicitly on the molecular interactions between the units of the system and on the amount of the impurities.[8] Consequently the obtained cooperative unit sizes depend on these factors in our case too.

Acknowledgement

We wish to thank Dr. C. Marzagora for computing assistance, W. van Osdol for useful discussions, Profs. I. Tarjan and G. Ronto for their stimulating support. This work was developed in the frame of the Italian-Hungarian Technical and Scientific Cooperation and was supported in part by an Italian CNR Public Health Project.

APPENDIX

Here we point out that Eqn. 1 can be derived for the size of the smallest subsystem too, if its partition function can be approximated by means of the maximum term.[14]

The partition function of the smallest independent subsystem is:

$$Q_{\mathrm{sub}} = \int_S (dS) \exp - \left[f(T,S) \cdot \mathcal{N}/kT \right] \tag{A1}$$

At the phase transition temperature, T_m there are two global maxima of the integrand at S_1 and S_2, i.e. $f(T_m, S_1) = f(T_m, S_2)$. Applying the maximum term approximation [14], Q_{sub} is approximated by the integrals around the maxima of the integrand:

$$\begin{aligned} Q_{\mathrm{sub}} &\approx \int_{S_1-\epsilon}^{S_1+\epsilon} (dS) \exp - [f\mathcal{N}/kT] + \int_{S_2-\epsilon}^{S_2+\epsilon} (dS) \exp - [f\mathcal{N}/kT] \\ &\approx 2\epsilon \left\{ \exp - \left[\mathcal{N} f(T,S_1)/kT \right] + \exp - \left[\mathcal{N} f(T,S_2)/kT \right] \right\} \end{aligned} \tag{A2}$$

where 2ϵ is the length of the small interval around S_1 or S_2. In this approximation the degree of phase transition in a subsystem, θ_{sub} is:

$$\theta_{\mathrm{sub}} \approx \bar{\theta} = \frac{\exp - \left[\mathcal{N} f(T,S_1)/kT \right]}{\exp - \left[\mathcal{N} f(T,S_1)/kT \right] + \exp - \left[\mathcal{N} f(T,S_2)/kT \right]} \tag{A3}$$

Since the subsystems are independent from each other θ_{sub} is equal to the degree of phase transition, θ for the whole system. The slope of the phase transition at the phase transition temperature is:

$$\begin{aligned}\left.\frac{d\theta}{dT}\right|_{T_\mathrm{m}} \approx \bar{\theta}(T_\mathrm{m})&\left\{-\frac{\mathcal{N}}{k}\left(\frac{1}{T_\mathrm{m}}\left.\frac{\partial f}{\partial T}\right|_{T_\mathrm{m},S_1}-\frac{f(T_\mathrm{m},S_1)}{T_\mathrm{m}^2}\right)\right\}\\ &-\bar{\theta}(T_\mathrm{m})\left[\bar{\theta}(T_\mathrm{m})\left\{-\frac{\mathcal{N}}{k}\left(\frac{1}{T_\mathrm{m}}\left.\frac{\partial f}{\partial T}\right|_{T_\mathrm{m},S_1}-\frac{f(T_\mathrm{m},S_1)}{T_\mathrm{m}^2}\right)\right\}\right.\\ &\quad+\left(1-\bar{\theta}(T_\mathrm{m})\right)\\ &\quad\left.\times\left\{-\frac{\mathcal{N}}{k}\left(\frac{1}{T_\mathrm{m}}\left.\frac{\partial f}{\partial T}\right|_{T_\mathrm{m},S_2}-\frac{f(T_\mathrm{m},S_2)}{T_\mathrm{m}^2}\right)\right\}\right] \qquad \text{(A4)}\end{aligned}$$

Since the specific enthalpy function is [14]:

$$h(T,S) = -T^2\frac{\partial(f/T)}{\partial T} = -T^2\left[\frac{1}{T}\frac{\partial f}{\partial T}-\frac{f}{T^2}\right] \qquad \text{(A5)}$$

and $\bar{\theta}(T_\mathrm{m}) = 1/2$ we obtain:

$$\begin{aligned}\left.\frac{d\theta}{dT}\right|_{T_\mathrm{m}} &\approx \frac{1}{2}\,\frac{\mathcal{N}h(T_\mathrm{m},S_1)}{kT_\mathrm{m}^2}-\frac{1}{4}\left[\frac{\mathcal{N}h(T_\mathrm{m},S_1)}{kT_\mathrm{m}^2}+\frac{\mathcal{N}h(T_\mathrm{m},S_2)}{kT_\mathrm{m}^2}\right]\\ &=\frac{1}{4}\,\frac{\mathcal{N}\left[h(T_\mathrm{m},S_1)-h(T_\mathrm{m},S_2)\right]}{kT_\mathrm{m}^2}=\frac{1}{4}\,\frac{\mathcal{N}\cdot\Delta H_\mathrm{cal}}{RT_\mathrm{m}^2} \qquad \text{(A6)}\end{aligned}$$

References

1. R. E. Jacobs, B. S. Hudson and H. C. Andersen, *Biochemistry* **16**, 4349 (1977).
2. H. L. Scott and W. H. Cheng, *Biophys. J.* **28**, 117 (1979).
3. E. Freire and B. Snyder, *Biochemistry* **19**, 88 (1980).
4. J. A. McCammon and J. M. Deutch, *J. Am. Chem. Soc.* **97**, 6675 (1975).
5. W. H. Cheng, *Biochim. Biophys. Acta* **600**, 358 (1980).
6. R. G. Priest, *Mol. Cryst. Liq. Cryst.* **60**, 167 (1980).
7. A. G. Lee, *Biochim. Biophys. Acta* **472**, 237 (1977).
8. S. Stankowski and B. Gruenewald, *Biophys. Chem.* **12**, 167 (1980).
9. I. P. Sugár and G. Monticelli, *Biophys. Chem.* **18**, 281 (1983).
10. S. Mabrey and J. M. Sturtevant, *Proc. Nat. Acad. Sci USA* **73**, 3862 (1976).

11. H. De Smedt, R. Borghgraef, F. Ceuterick and K. Hermans, *Biochim. Biophys. Acta* **556**, 479 (1979).
12. H. J. Hinz and J. M. Sturtevant, *J. Biol. Chem.* **247**, 6071 (1972).
13. B. H. Zimm and J. K. Bragg, *J. Chem. Phys.* **31**, 526 (1959).
14. T. L. Hill, *Statistical Thermodynamics*, (Addison-Wesley, U.S.A., 1960), and *Thermodynamics of Small Systems* (Benjamin, New York, 1963, 1964).
15. N. Albon and J. M. Sturtevant, *Proc. Natl. Acad. Sci. USA* **75**, 2258 (1978).
16. S. Doniach, *J. Chem. Phys.* **68**, 4912 (1978).
17. E. A. Guggenheim, *Thermodynamics* (North-Holland, Amsterdam, N.Y. Oxford, 1977).