Role of soft and hard aggregates in the thermodynamics of lipid dispersions

Carla Goldman, Karin A. Riske, and M. Teresa Lamy-Freund
Departamento de Física Geral, Instituto de Física, Universidade de São Paulo, C.P. 66318, 05315-970 São Paulo, SP, Brazil

(Received 15 March 1999)

We study the thermodynamics of a two-dimensional polydisperse ideal gas model of different species of aggregates. We show that if these aggregates are distinguished not only by their sizes but also by their ability to display shape fluctuations, the system presents dominance of one or other species, depending on the temperature region. This result, which emerges solely from the statistics of the model in total absence of interaggregate interactions, describes well the observed temperature dependence of light scattering in dispersions of dimyristoyl phosphatidylglycerol, a negatively charged lipid. [S1063-651X(99)13512-8]

PACS number(s): 87.16.Dg, 82.60.Lf, 82.20.Db, 82.70.−y

I. INTRODUCTION

In aqueous medium and at relative low concentrations, DMPG (dimyristoyl phosphatidylglycerol), a negatively charged lipid, aggregates into vesicles of various sizes and shapes. Considering that lipid vesicles have been widely used as model systems for cell membranes, the thermotropic properties of such systems are of great biological interest regarding for instance, the state of the lipid matrix that favors peptide-lipid interactions. For this, DMPG dispersions have been investigated using diverse experimental techniques including differential scanning calorimetry (DSC) [1], electron paramagnetic resonance (EPR), and light scattering (LS) [2]. Worth noticing, reports from DSC and LS indicate two abrupt changes in the behavior of observed properties under variation of the temperature T of the system. In particular, LS data taken at various lipid concentrations and ionic strengths show that at $T=T_m \sim 20\,^\circ\mathrm{C}$ the observed turbidity τ of DMPG dispersions decreases abruptly from a relatively high level τ_0 and stay at a relative low level τ_1 up to $T=T_{\mathrm{post}} \sim 35\,^\circ\mathrm{C}$, where it increases sharply up to saturation at τ_2, slightly below τ_0 [2] (see Fig. 1). It is noticeable that a similar behavior for τ has already been seen in certain quaternary mixtures of SDS-water-pentanol dodecane [3]. For these, data have been rationalized under the perspective of critical phenomena in terms of a high temperature three-dimensional (3D) Ising critical point, and a low temperature mean-field critical point related to a transition from a micellar homogeneous phase to a spongelike phase [4]. A necessary condition for that picture though, is the presence of attractive interactions among micelles which, in the context, are expected to increase with temperature [3].

As discussed in Ref. [2], there are no indications in the relevant data from DMPG of the existence of attractive interactions among the aggregates formed, as one should expect for the very diluted regimes considered in those experiments. Moreover, because T_m is almost coincident to the temperatures at the main transition undergone by membrane lipid bilayers of DMPG, it appears natural in principle, the suggestion in Ref. [2] of relating the change in the turbidity at $T=T_m$, which can also be monitored by DSC and EPR, to the melting of lipid hydrocarbon chains. Within this picture, however, there is not yet a consensus on the origins of the abrupt increasing of DMPG turbidity observed at $T=T_{\mathrm{post}}$, generally ascribed to an unconventional phase change of the system named the post transition [2,5]. Additional data obtained by DSC are also sensitive to T_{post} whereas data by EPR are not. As it is shown in Refs. [1] and [2] T_{post} is mostly modified by the ionic strength of the dispersion and/or by its lipidic content in the low concentration range. There are also in Ref. [2] some evidence from Zimm plot analysis indicating that molecular weights of the lipidic structures are smaller in the low turbidity region than those of typical aggregates in the regions of temperatures below T_m and above T_{post}.

Here, we show that the qualitative behavior of τ at the experimental conditions of Fig. 1 can emerge solely from the statistics of a model dispersion consisting essentially of a two-dimensional mixture of distinct forms (species) of lipid aggregates (droplets) in chemical equilibrium, but under total absence of interaggregate interactions. This model comprises minimum size structures containing a definite number of lipids, say, N_0; these small aggregates in turn, are identified as the model monomers that compose any other ''droplet'' of arbitrary size. The essential ingredient of this model though, is the fact that these large aggregates are distinguished not only by their sizes but also by their ability to display shape

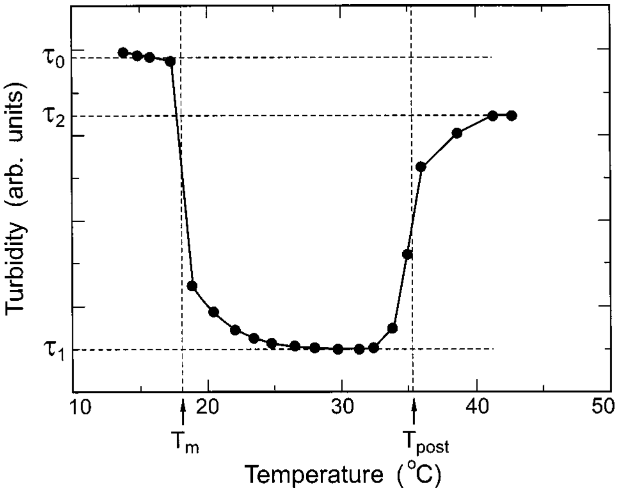

FIG. 1. Temperature dependence of 90° light scattering of dispersions of 10 mM DMPG in 10 mM Hepes pH 7.4 with no added salt, from Ref. [2].

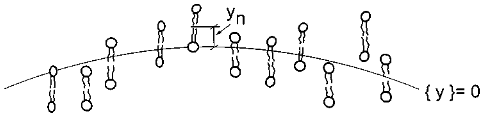

FIG. 2. Model vesicle.

(thermal) fluctuations. Accordingly, in addition to hard aggregates which have only translational degrees of freedom in the entire volume V of the system, there are also present in the model other structures, i.e., soft aggregates whose constituting monomers undergo small displacements from their equilibrium positions on aggregate's ''surface.'' The thermodynamics of such a multicomponent ideal mixture is then disclosed by the analysis of the convergence of a constraint equation set up to fix the total density of lipids present in the dispersion to a given experimental value ρ_{expt}.

Because we do not point to particular processes underneath formation or dissolution of aggregates, this analysis is restricted to the statistical aspects of the model dispersion, irrespective of the nature of direct or induced interactions among lipids that lead to their aggregation. In spite of this, the results obtained from this analysis suggest that the main features displayed in Fig. 1 can indeed be predicted in this way, the behavior of turbidity being associated with the dominance in the system of one or other species considered, depending on temperature: hard aggregates prevail at $T < T_m$ and soft aggregates prevail at $T > T_{\mathrm{post}}$ whereas in the range within $T_m < T < T_{\mathrm{post}}$, there is dominance of monomers.

II. MODEL FOR SOFT AGGREGATES

We distinguish soft from hard aggregates of the same size by their energy contents. For an isolated plane vesicle of very small curvature with s monomers, this difference is microscopically modeled by the internal Hamiltonian

$$H_{\mathrm{int}}^{(s)} = \sum_{n=1}^{s} \left\{ \frac{1}{2} m \dot{y}_n^2 + \frac{1}{2} K (y_n - y_{n-1})^2 + V(y_n;\alpha) \right\} \tag{1}$$

with periodic boundary conditions. In this, we consider only one internal degree of freedom at radial direction for each constituting monomer so that y_n and $\dot{y}_n$ are, respectively, the position and momentum, with respect to the equilibrium site, of the n sequence labeled monomer on the aggregate's ''surface'' (see Fig. 2). m is the total mass of a monomer; K and α are model parameters. The form of Eq. (1) reflects the general tendency of hydrophobic molecules to stay parallel to each other on the aggregate [6]. This is modeled by the elastic contribution in the second term on the right-hand side of Eq. (1), whereas the local potential

$$V(y_n) = \alpha y_n^2 \tag{2}$$

is essential for model stability [7,8].

III. MODEL DISPERSION

Consider now a multicomponent ideal gas, immersed in a 2D volume V, that consists on a collection of monomers coexisting in chemical equilibrium with hard and soft aggregates of all sizes. The internal degrees of freedom of the soft kind are described according to the Hamiltonian (1). This model system shall be used here to describe some features of the behavior of DMPG dispersions observed by LS in Ref. [2]. In order to proceed in the analysis, we construct the grand canonical partition function of the problem $\Xi = \Xi_{\mathrm{soft}} \Xi_{\mathrm{hard}}$ which accounts for a contribution Ξ_{soft} from soft aggregates

$$\Xi_{\mathrm{soft}}(\{\tilde{\mu}_s\}; V, T) = \prod_{s=2}^{\infty} \exp(\tilde{q}_s \tilde{z}_s) \tag{3}$$

as well as for a similar contribution Ξ_{hard} from hard aggregates

$$\Xi_{\mathrm{hard}}(\{\mu_s\}; V, T) = \prod_{s=1}^{\infty} \exp(q_s z_s), \tag{4}$$

where

$$q_s = sV/\Lambda^2 \tag{5}$$

and

$$\tilde{q}_s = q_s \tilde{q}_{\mathrm{int}}(s). \tag{6}$$

The quantities q_s and $\tilde{q}_s$ are, respectively, the (canonical) partition functions for hard and soft aggregates with s monomers each (the number of monomers defines the size of the aggregate). Also, $\Lambda = (2\pi m k_B T)^{-1/2}$, $\beta = 1/k_B T$, and

$$\tilde{q}_{\mathrm{int}}(s) = \frac{1}{\Lambda^s} \int_{-\infty}^{\infty} \prod_{n=1}^{s} dy_n e^{-\beta H_{\mathrm{int}}^{(s)}(\{y_n\})}, \tag{7}$$

where $H_{\mathrm{int}}^{(s)}$ is given by Eq. (1). In addition, $z_s = e^{\beta \mu_s}$ and $\tilde{z}_s = e^{\beta \tilde{\mu}_s}$ are the activities with μ_s and $\tilde{\mu}_s$ the corresponding chemical potentials for hard and soft aggregates, respectively.

Now, observe that (i) μ_s and $\tilde{\mu}_s$ assume only negative values in the ideal gas limit, as considered here, (ii) free monomers are accounted by q_s, with $s = 1$, and (iii) due to the assumed chemical equilibrium, one has $\tilde{z}_s = z_s = z^s$ where $z = e^{\beta \mu}$ is the activity and μ the chemical potential of a monomer. One then determines the equilibrium density of monomers that remain free in dispersion

$$\rho_1(\mu, T) = \frac{z}{\Lambda^2} \tag{8}$$

and also the equilibrium densities of monomers partitioned into hard aggregates, $\rho_{\mathrm{hard}}(\mu, T) = (1/V) \Sigma_{s=2}^{\infty} s q_s z^s$ and into soft aggregates, $\rho_{\mathrm{soft}}(\mu, T) = (1/V) \Sigma_{s=2}^{\infty} s \tilde{q}_s z^s$, at given temperature and chemical potential.

The computation of $\tilde{q}_{\mathrm{int}}$ in Eq. (7) is performed in Ref. [9] and the result is written as a sum over all eigenvalues λ_p of a transfer matrix Υ defined by

$$\langle n | \Upsilon | n-1 \rangle = \exp\left\{ -\beta \left[\frac{K}{2} (y_n - y_{n-1})^2 + \frac{\alpha}{2} (y_n^2 + y_{n-1}^2) \right] \right\}.$$

One finds that

$$\tilde{q}_{\rm int}(s)=\frac{1}{\Lambda^s}\sum_{p=0}^{\infty}\lambda_p^s \tag{9}$$

with

$$\lambda_p=2\sqrt{\frac{\pi}{\beta(K+\alpha+h)}}\,C^p \quad \text{for} \quad p=0,1,2,\ldots, \tag{10}$$

where $C=K/(K+\alpha+h)$ and $h=[(K+\alpha)^2-K^2]^{1/2}$.

For $\alpha \gg K$, or $C \ll 1$, we take for $\tilde{q}_{\rm int}(s)$ the contribution from the largest eigenvalue λ_0 of Υ. With this, the partition function of a soft aggregate simplifies to

$$\tilde{q}_s \simeq (\Omega T)^s q_s, \tag{11}$$

where q_s is given in Eq. (5) and Ω is a constant defined by

$$\Omega \equiv \left(\frac{8\pi^2 m k_B^2}{K+\alpha+h}\right)^{1/2}. \tag{12}$$

Under these conditions, we find

$$\rho_{\rm hard}(\mu,T)=\frac{z}{\Lambda^2}\left[\frac{(1+z)}{(1-z)^3}-1\right], \tag{13}$$

with $z<1$, and

$$\rho_{\rm soft}(\mu,T)=\frac{\sigma}{\Lambda^2}\left[\frac{(1+\sigma)}{(1-\sigma)^3}-1\right], \tag{14}$$

with $\sigma<1$, where σ is defined by

$$\sigma=\frac{\lambda_0}{\Lambda}z=\Omega T z. \tag{15}$$

In the following, we analyze the consequences of the results derived above to the thermodynamical properties of the model.

IV. ANALYSIS OF THE MODEL

Equation (11) allows for an expression for the Helmholtz free-energy of a soft aggregate, $F_{\rm soft}^{(s)}=-k_B T \ln \tilde{q}_s$, which can readily be compared with corresponding quantity $F_{\rm hard}^{(s)}=-k_B T \ln q_s$ of a hard aggregate of the same size *s*:

$$F_{\rm soft}^{(s)}=F_{\rm hard}^{(s)}-k_B T s \ln(\Omega T). \tag{16}$$

For $T<\Omega^{-1}$, $F_{\rm soft}>F_{\rm hard}$ so that low temperatures favor hard aggregates, whereas for $T>\Omega^{-1}$, $F_{\rm soft}<F_{\rm hard}$ meaning that high temperatures favor soft aggregates [10].

The above argument is commonly used to examine stability conditions of a system presenting more than one thermodynamical phase. Such an argument, however, does not justify the *a priori* presence of hard aggregates in the system, as stated by model construction. The justification comes from the requisite that the following constraint:

$$\rho_b(\mu_{\rm expt},T)=\rho_{\rm expt}/N_0 \tag{17}$$

be resolved for at all temperatures and for all values of lipid concentration $\rho_{\rm expt}$, at a given experimental condition in the low concentration range. The quantity $\rho_b(\mu,T)$ entering Eq. (17), is defined as the sum of the partial densities derived above:

$$\rho_b(\mu,T)=\rho_1(\mu,T)+\rho_{\rm hard}(\mu,T)+\rho_{\rm soft}(\mu,T). \tag{18}$$

The constraint (17) fixes the chemical potential at a value $\mu_{\rm expt}$ such that $\rho_b(\mu_{\rm expt},T)$ coincides with the total density of monomers $\rho_{\rm expt}/N_0$ present in the model dispersion.

We proceed on the analysis of Eq. (17) based on the arguments used in Ref. [11]. We shall show that at each region of temperature the solution to Eq. (17) is saturated by one and only one of the components in the right-hand side of Eq. (18). We shall then make an attempt to relate this fact to the observed behavior of the turbidity in DMPG dispersions.

For this, notice that both Eqs. (13) and (14) are monotone increasing functions of μ such that $\lim_{\mu\nearrow\bar{\mu}_{\rm hard}}\rho_{\rm hard}\to\infty$ and $\lim_{\mu\nearrow\bar{\mu}_{\rm soft}}\rho_{\rm soft}\to\infty$, where

$$\bar{\mu}_{\rm hard}=0 \tag{19}$$

and

$$\bar{\mu}_{\rm soft}=-k_B T\ln(\Omega T). \tag{20}$$

Notice also that $\lim_{\mu\nearrow\bar{\mu}_{\rm hard}}\rho_{\rm soft}$ and $\lim_{\mu\nearrow\bar{\mu}_{\rm soft}}\rho_{\rm hard}$ are finite quantities, and that for $T<\Omega^{-1}$, $\bar{\mu}_{\rm soft}>\bar{\mu}_{\rm hard}$, whereas for $T>\Omega^{-1}$, $\bar{\mu}_{\rm soft}<\bar{\mu}_{\rm hard}$. It thus seems natural to define a "critical temperature" $T_C=\Omega^{-1}$ and observe that by increasing the chemical potential, approaching zero from negative values at constant temperature *T*, there is always a solution to Eq. (17) in either one of two conditions (see Fig. 3). (i) if $T<T_C$ and $\rho_{\rm expt}\gg\tilde{\rho}_{\rm max}(T)\equiv\rho_1(\bar{\mu}_{\rm hard},T)+\rho_{\rm soft}(\bar{\mu}_{\rm hard},T)$, then Eq. (17) is saturated by hard aggregates meaning that the number density of monomers which takes part into hard aggregates dominates over the remaining monomers. Or, (ii) if $T>T_C$ and $\rho_{\rm expt}\gg\rho_{\rm max}(T)\equiv\rho_1(\bar{\mu}_{\rm soft},T)+\rho_{\rm hard}(\bar{\mu}_{\rm soft},T)$, then Eq. (17) is saturated by soft aggregates.

As can be seen in Fig. 3, for $T<T_c$ the curves $\rho_1(\mu,T)$ and $\rho_{\rm hard}(\mu,T)$ intercept each other at a certain value of μ, whereas for $T>T_c$ the curves $\rho_1(\mu,T)$ and $\rho_{\rm soft}(\mu,T)$ intercept each other at a different value of μ. This fact enables us to introduce a (temperature dependent) critical concentration $\rho_c(T)$ such that $\rho_c(T)=\rho_b(\mu_c(T),T)$, where the (critical) chemical potential $\mu_c(T)$ depends on the temperature and it is defined in such a way to coincide with the solution of either

$$\rho_1(\mu_c,T)=\rho_{\rm hard}(\mu_c,T) \tag{21}$$

within the region where $T<T_C$ or

$$\rho_1(\mu_c,T)=\rho_{\rm soft}(\mu_c,T) \tag{22}$$

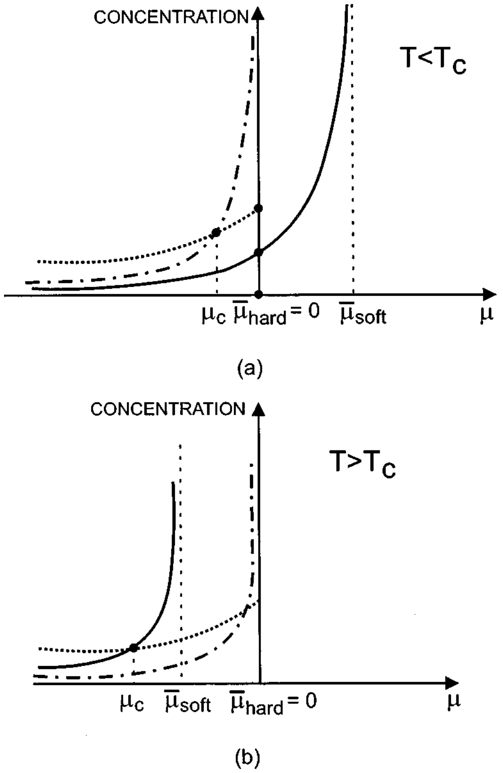

FIG. 3. Graphical representation of Eq. (17) in different regions of temperature. (a) $T<T_C$; (b) $T>T_C$. In both diagrams, ρ_1 is represented by a dashed line, ρ_{soft} by a solid line, and ρ_{hard} by a dash-dotted line.

within the complementary region where $T>T_C$. The solutions to the above equations are represented in Fig. 4 and are given explicitly by

$$(a)\quad \rho_c(T<T_c)\simeq\frac{2}{\Lambda^2}z_c, \tag{23}$$

where

$$z_c=z[\mu_c(T<T_c)]\simeq 0.126 \tag{24}$$

and

$$(b)\quad \rho_c(T>T_c)\simeq\frac{2}{\Omega T\Lambda^2}\sigma_c, \tag{25}$$

where

$$\sigma_c=\sigma(\mu_c(T))=1+3(\theta^2-1)^{1/3}[(\theta-1)^{1/3}-(\theta+1)^{1/3}] \tag{26}$$

and θ has an explicit dependence on the temperature, given by

$$\theta=\left[1+\frac{1}{27[1+(\Omega T)^{-1}]}\right]^{1/2}. \tag{27}$$

Moreover, one sees from definition (15) and Eqs. (13) and (14) that at $T=T_c$, $\rho_{\text{hard}}(\mu_c(T_c),T_c)=\rho_{\text{soft}}(\mu_c(T_c),T_c)\equiv\frac{1}{3}\rho_c^*$. This defines the quantity ρ_c^* which is also indicated in Fig. 4. Next, we show why the above analysis suffices for an interpretation of the LS data in Fig. 1.

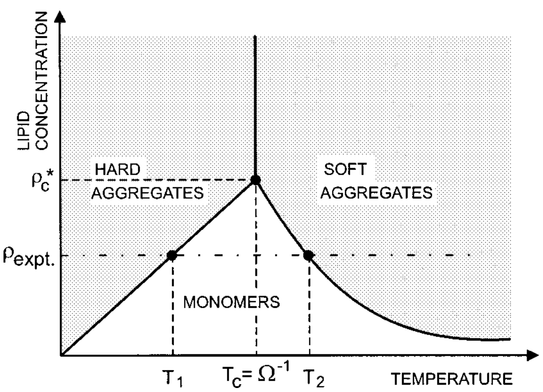

FIG. 4. Phase diagram of the model. The lines represent the solutions of Eq. (21) for $T<T_C$ and Eq. (22) for $T>T_C$.

V. DISCUSSION

Because the model discussed here does not exhibit sharp phase transitions since all thermodynamical functions are analytical at all values of model parameters, we cannot assert on its use to describe critical behavior eventually displayed by dispersions of DMPG. For our purposes, however, the model suffices to characterize the different regions of Fig. 1, far from eventual transition temperatures, in terms of three distinct phases that are regulated by the distribution of monomers into the different species considered.

In fact, it can be observed in the diagram of Fig. 4 that equation $\rho_{\text{expt}}=\rho_c(T)$ possesses two solutions (one at $T=T_1$ and another at $T=T_2$) for all values of total lipid concentration in the range $\rho_{\text{expt}}<\rho_c^*$. To relate this fact with the observed behavior of turbidity in DMPG, we put $T_m\equiv T_1$ and $T_{\text{post}}\equiv T_2$, where T_m and T_{post} coincide, respectively, with the experimental temperatures indicated in Fig. 1, at the considered values of lipid concentrations. Under these conditions, one can ascribe the observed regions of high turbidity at $T<T_m$ and at $T>T_{\text{post}}$ shown in Fig. 1 to the dominance in each of these regions of the experimental systems, of hard and of soft aggregates, respectively. Accordingly, the intermediate region of low turbidity presents dominance of free monomers (or minimum size aggregates) of DMPG, in agreement to the data from Zimm plot shown in Ref. [2].

These results should be understood in the same context as the usual theoretical approaches to study the onset of the critical micelle concentrations (CMC) in amphiphilic dispersions [12]. In these, an *a priori* presence of polydisperse (hard) aggregates and free monomers is also assumed. It is then shown the conditions by which the density of amphiphiles taking part in aggregates is dominant at low temperatures $(T<T_1)$. In the present case, fluctuations of soft aggregates introduce additional contributions both to the energy and to the entropy of the system. Our results suggest that the combined (entropic) contributions due to both polydispersity and fluctuations are sufficient to justify the presence of aggregates (of the soft kind) even at high temperatures $(T>T_2)$. There are no indications, however, that either contributions considered individually can overcome the entropy of monomers in this region.

We should also comment on the tentative assignment of T_m (and thus T_1) to the temperature at the main lipid transition. Actually, the main transition has been characterized in individual lipid bilayers and associated to the appearance of a certain degree of mobility of the constituting hydrocarbon chains. Such mobility might be associated to the fluctuations considered in the context of our model. However, the main transition, as is known, preserves the average molecular weight of the large structures (membranes). For the model systems considered here we have shown that if $\rho_{\mathrm{expt}}<\rho_c^*$, such phenomenon is not expected to play a relevant role since by increasing the temperature the system passes through an intermediate region within $T_m<T<T_{\mathrm{post}}$, where the mixture sustains mostly monomers. However, if $\rho_{\mathrm{expt}}>\rho_c^*$, then $T_1=T_2=T_c$ and we recognize that if lipid concentrations are sufficiently high, our theoretical model indeed describes a phenomenon related to a direct change from hard to soft aggregates, which is quite similar to the one observed at the main (or gel to liquid crystal) phase transition in bilayers.

Therefore, the analysis of the model focusing only on the statistics of the mixture allowed us to obtain, in a very simple way, qualitative insights towards a distinct interpretation of LS data of DMPG dispersions. We believe that such approach can be extended to rationalize other physical properties of these systems (e.g., the dependence of LS data on the ion content of solution) that are also under investigation in the literature [2,5]. Moreover, the approach introduced here might be useful for analyzing whether and under what conditions fluctuations on the surface of lipidic structures in dispersions are of some relevance to control their permeability to external agents, a current problem regarding, for instance, the development of drug carrying liposomes devices in biological systems.

ACKNOWLEDGMENTS

This work was supported by USP, FAPESP, CNPq, and FINEP.

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[7] The model discussed here parallels continuous versions in which contributions from curvature deviations to the Hamiltonian are usually approximated by quadratic (elastic) terms [8]. It is worth mentioning, however, that by being a microscopic and exactly solvable model, our description allows one to disclose not only the properties of a single aggregate seen as a thermodynamical subsystem, but also the properties of a polydisperse solution of such subsystems, which, as we show here, are essential for interpretation of experimental data.

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[10] Because the competition between $F_{\mathrm{hard}}^{(s)}$ and $F_{\mathrm{soft}}^{(s)}$ is controlled by a logarithm function of temperature, one expects that the qualitative results presented here would be preserved in considering the model Hamiltonian (1) in higher dimensions. In fact, dimensionality effects can be estimated in this case using scaling analysis: the diverse degrees of freedom contribute with one power of T each to the argument of the logarithm. Eventually, one could predict modifications on the dependence of Ω on the model parameters which, however, are of no relevance to the aspects of the model upraised here.

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