

Thermodynamic and Textural Characterization of DPPG Phospholipid Monolayers

D. Vollhardt,*[†] V. B. Fainerman,[‡] and S. Siegel[†]

Max-Planck-Institut für Kolloid- und Grenzflächenforschung, D-14424 Potsdam, Germany, and International Medical Physicochemical Center, Donetsk Medical University, 16 Ilych Avenue, Donetsk 340003, Ukraine

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The phase properties of DPPG (dipalmitoyl-phosphatidylglycerol, sodium salt) monolayers are investigated at different temperatures $25\text{ }^{\circ}\text{C} \leq T \leq 34\text{ }^{\circ}\text{C}$ where the surface pressure–area (Π – A) isotherms have large two-phase coexistence regions. The studies are based on the thermodynamic and textural characterization of the monolayers. The main phase transitions obtained from the Π – A isotherms are in complete agreement with the Brewster angle microscopy results. In equilibrium, compact domains are formed which are never really circular. The domains differ from each other in the azimuthal tilt, but they have no inner texture. Over the entire region of the gaseous to the condensed state, the experimental Π – A isotherms are well described by an equation of state recently derived on the basis of the generalized Volmer's equation and the quasi-chemical equilibrium model of 2D aggregation. The possible dissociation effect on the monolayer properties of the ionic DPPG can be largely ignored, as clarified by extending the theoretical approach under consideration of the dissociation degree of the monolayer substance. The standard thermodynamic characteristics of the 2D aggregation are calculated for different temperatures. The analysis of these results shows that, unlike the micelle formation, within the Langmuir monolayer the aggregation of the amphiphilic molecules to condensed phase results only in an entropy decrease of the system due to the ordering of the amphiphilic molecules.

Introduction

Model monolayers at the air–water interface of various phospholipids have been intensively studied for obtaining information on specific processes in biological systems. In recent years, wide interest is focused on the processes of the alveolar lining layer in lungs. For a better understanding of the multicomponent alveolar lining layer, it is of primary interest to characterize the monolayer properties such as thermodynamics, phase behavior, structure, texture, and stability of the relevant main phospholipids. Considerable progress has been made in the understanding of the fundamental monolayer properties of the main phospholipid DPPC (dipalmitoyl-phosphatidylcholine),^{1–5} whereas corresponding information on DPPG (dipalmitoyl-phosphatidylglycerol) which is also one of the major components of the lung surfactant^{6–14} are not yet available.

For different uncharged membrane lipids, it was found that shape and texture of the condensed phase domains are very different depending on the molecular structure of the polar headgroup.^{15,16} On the other hand, there is less information whether or in which way the monolayer properties of the ionic DPPG deviate from those of the uncharged lipid monolayers. The situation is made more complicated by the fact that some conclusions drawn in previous papers are based on surface pressure (Π)–area (A) isotherms which cannot represent real monolayer data as the area values of the compressed monolayers are much smaller than the molecular cross section of a DPPG molecule.^{17,18}

Therefore, the objective of the present paper is to provide a reliable characterization of the thermodynamic and textural

properties of DPPG monolayers. Our main interest is focused on the phase properties of the DPPG monolayers based on their thermodynamic and textural characterization. These studies are performed at different temperatures where the Π – A isotherms have large two-phase coexistence regions. An equation of state valid for the bimodal distribution of the monolayer material is used for the theoretical description of the phase behavior of the monolayers. For the discussion of the results, the standard thermodynamic characteristics of the 2D aggregation are determined.

Materials and Methods

The surface pressure was measured using a computer-interfaced film balance with a Wilhelmy-type surface pressure measuring system.¹⁶ The surface pressure–area (Π – A) isotherms of DPPG monolayers spread on pure water, were measured at different temperatures between 22 and 36 $^{\circ}\text{C}$. The isotherms were recorded at a constant compression rate $r = 1/A \text{ d}A/\text{d}t$ instead of at a constant barrier velocity. Simultaneous imaging of the monolayers was performed with a Brewster angle microscope (BAM 2, NFT, Göttingen) coupled to the film balance. The microscope is equipped with a special scanning technique for providing sharp images. To obtain BAM images real in scale and angle, the CCD sensor of the camera is tilted according to the Scheimpflug condition. The application of a green laser (Uniphase, San Jose, CA) allows a resolution of the BAM of approximately 3 μm .

DPPG and DPPC ($\geq 99\%$ purity) were purchased from Sigma (Deisenhofen, Germany) and used without further purification, just as were the spreading solvents chloroform (p.a. grade), received from Baker, Deventer, Holland and methanol (p.a. grade), obtained from Merck, Darmstadt, Germany. DPPG was spread from a 10^{-3} M chloroform/methanol (1/3, v/v) solution, DPPC from a 10^{-3} M chloroform solution.

* Corresponding author.

[†] Max-Planck-Institut für Kolloid- und Grenzflächenforschung.

[‡] International Medical Physicochemical Center.

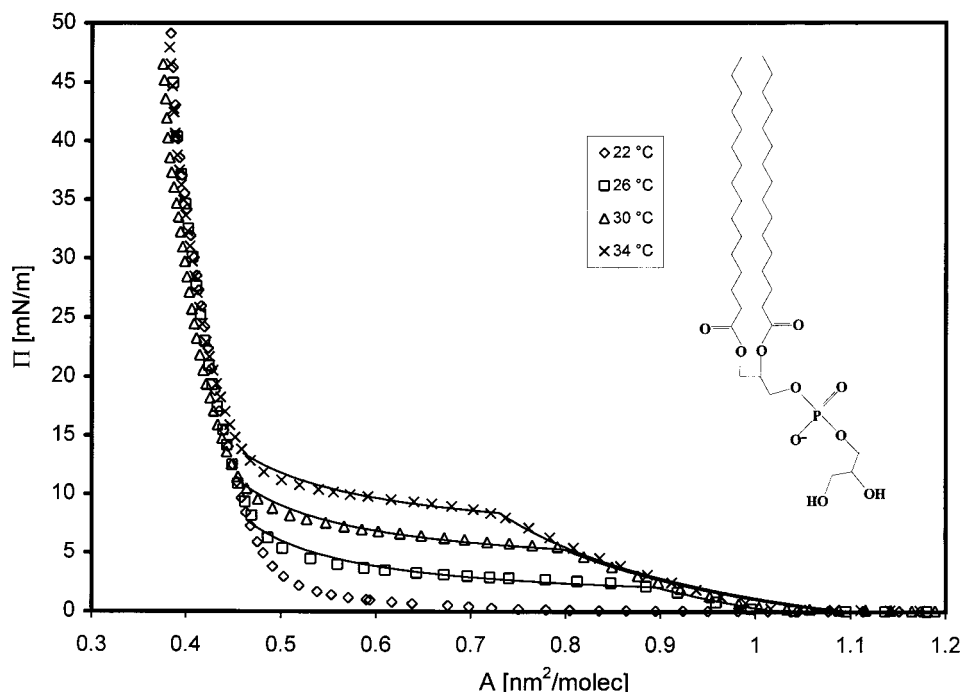


Figure 1. Experimental and theoretical Π – A isotherms of DPPG monolayers at different temperatures. The values of the parameters in eqs 1–4 are shown in Table 1. Pressure at phase transition in dependence on the temperature.

The water used as subphase for the experiments (pH = 5.8) was made ultrapure by a Millipore desktop unit (Millipore, Eschborn, Germany).

Results and Discussion

The approach based on the combination of the generalized Volmer's equation¹⁹ and the quasi-chemical equilibrium model of 2D aggregation in monolayer²⁰ was developed in ref 21. It was assumed that the area per one molecule in the aggregate can differ from the area per free monomer molecule. Also, the condition which describes the equilibrium between the monomers and aggregates was formulated with due account for the free surface existing in the monolayer.

For the region $A < A_c$, the following equation of state valid for the bimodal distribution (large clusters and monomers) holds²¹

$$\Pi = \frac{RT(A/A_{c\Pi})\beta}{A - \omega[1 + \epsilon((A/A_{c\Pi})\beta - 1)]} - B \quad (1)$$

where

$$\beta = 1 + \frac{\omega(1 - \epsilon)}{A_{c\Pi}} - \frac{\omega(1 - \epsilon)}{A} \quad (2)$$

$$A_{c\Pi} = A_c \exp\left(\frac{(\Pi - \Pi_c)\epsilon\omega}{RT}\right) \quad (3)$$

$$\epsilon = 1 - \omega_{(n)}/\omega \quad (4)$$

where Π is the surface pressure, A the current area per one molecule, R the gas constant, T the temperature, ω the area per one mole of surfactant in the extremely compressed gaseous monolayer, $\omega_{(n)}$ the area per one mole of monomers in a cluster, B a constant, A_c the area per molecule of the main phase transition point at which the 2D transition commences i.e., at $\Pi = \Pi_c$. For $A = A_c$, eqs 1–3 can be transformed in the

ordinary Volmer's equation,²² which is carried out in the region $A \geq A_c$.

$$\Pi = \frac{RT}{A - \omega} - B \quad (5)$$

Therefore, the equation of state (eq 1) involves four constants (B , A_c , ω , and ϵ) and describes the entire region of surface pressure, from gaseous monolayer to the condensed state. Two constants (B and ω) are applicable to the gaseous monolayer region. The value A_c is the coordinate of the break point of the Π – A isotherm located at the beginning of the phase transition region. Only the parameter ϵ refers explicitly to the 2D phase transition region, and affects additionally the isotherm shape at $A < A_c$.

The experimental Π – A isotherms (for the area per one molecule) of DPPG monolayers at various temperatures are shown in Figure 1. It is seen that the pure fluid (gaseous) phase of the monolayer of this dissociated phospholipid does not occur at temperatures below 22 °C. Obviously, two-dimensional condensation of the DPPG monolayer exists at molecular area values of about 1 nm², so that the monolayer is already in the state of the two-phase coexistence region at a surface pressure of about zero. At higher temperatures of ≥ 25 °C, however, the Π – A isotherms demonstrate that the formation of the condensed phase occurs at real surface pressures. Figure 2 shows the linear dependence of the surface pressure of the main phase transition point, Π_t , on the temperature. At $A > A_c$ the monolayer is in the fluid (gaseous) state, e.g., it extends up to 0.73 nm² at the highest measured temperature of 34 °C (Figure 1).

The Π – A isotherms of the DPPG monolayers are in complete agreement with the results of the BAM studies. At compression, regularly shaped domains are only formed in the two-phase coexistence region after the main phase transition point in the temperature range between 26 °C and 37 °C. If the condensed phase exists already at zero surface pressure after spreading the monolayer material ($T < 25$ °C), the conditions of spreading and solvent evaporation affect nucleation and growth of the

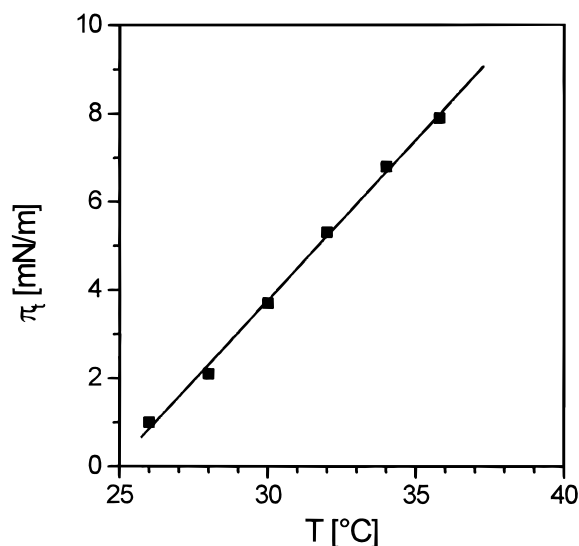


Figure 2. Surface pressure of the main phase transition point of DPPG monolayers in dependence on temperature.

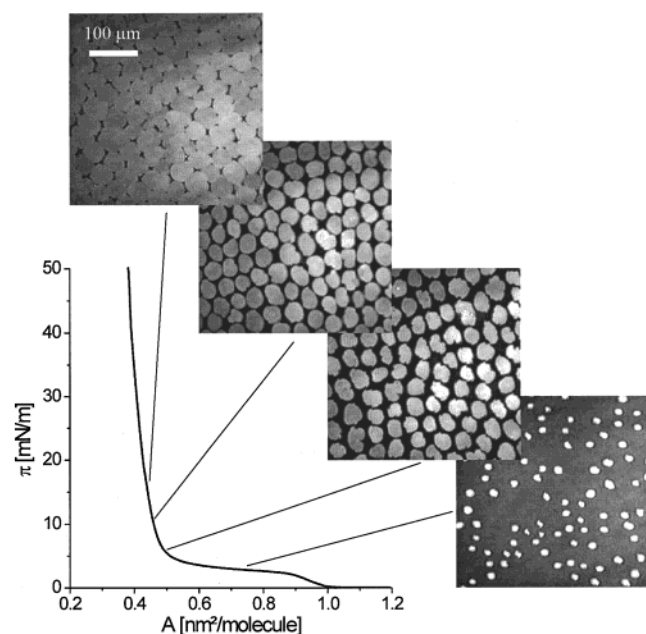


Figure 3. Typical BAM images for selected stages of the Π - A isotherm at 26 °C. Compression rate: $1.25 \times 10^{-3} \text{ s}^{-1}$.

condensed material and only irregularly shaped textures are developed. Figure 3 shows a sequence of typical BAM images for a selected Π - A isotherm at 26 °C. First small condensed phase domains appear at areas smaller than A_c . At further compression, the domains grow within the "plateau" region of the isotherm to an average size of about 50–60 μm in diameter. The similar size of the domains indicates that most of them are formed simultaneously after the main phase transition point. The compact domains are not regularly shaped, nor are they really round. Therefore, the line tension seems to be rather small. An inner texture does not occur. At the end of the "plateau" of the isotherm the domains are rather tightly packed without touching each other. On further compression, the domains impinge increasingly on each other with increasing surface pressure. Despite the rather low line tension, the domain fusion occurs only at relatively high pressure. Although the domains do not have an inner texture, their molecules are obviously tilted. This is in agreement with the BAM results which reveal that the molecules within one domain are homogeneously reflecting,

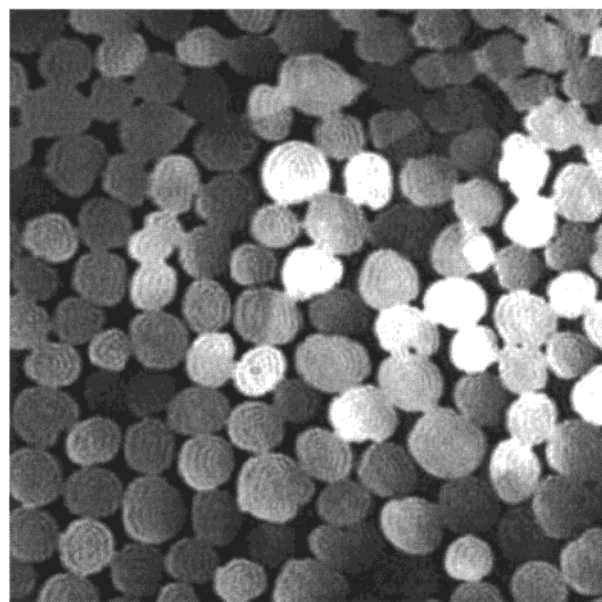


Figure 4. BAM image of compact condensed phase domains of DPPG monolayers at the analyzer position of 60°. The different reflectivity of the homogeneous reflecting domains indicates a different azimuthal tilt. $T = 26 \text{ °C}$, $A = 0.441 \text{ nm}^2$, $\Pi = 15 \text{ mN m}^{-1}$.

but the respective domains reflect differently (Figure 4). That means the molecules of one domain have the same azimuthal tilt.

Some domains exist which reflect with two different azimuthal tilt angles. They are obviously formed by merging two domains that reflect differently.

However, the azimuthal tilt of different domains is different. Recent GIXD (grazing incidence X-ray diffraction) studies of DPPG monolayers corroborate the result that the DPPG molecules are tilted.²³ At low surface pressures the DPPG molecules are strongly tilted (at 5 mN m^{-1} a polar tilt angle of $\sim 26^\circ$). With increasing surface pressure Π , the polar tilt angle decreases and at $\Pi = 30 \text{ mN m}^{-1}$ the molecules are perpendicularly oriented. These results on the ordering of the DPPG domains suggest that the merging of the domain is inhibited by the differences in the azimuthal tilt of the domains.

On the other hand, the rather low line tension facilitates the development of fingered nonequilibrium shapes with increasing compression rate (Figure 5). The monolayer deviates increasingly from equilibrium with increasing compression rate so that, correspondingly, more fingered domains are formed.

The experimental Π - A isotherms can be theoretically described by eqs 1–4. The solid lines in Figure 1 show calculated isotherms. Table 1 summarizes the corresponding parameters which were determined for various temperature values. It can be seen that the calculated results are in reasonable agreement with experimental data (with average deviation between the calculated and measured data of about 0.3 mN/m). The value per one DPPG molecule within the aggregate, 0.36–0.39 nm^2 , as estimated from the fitting program, agrees well with minimum values per one DPPG molecule in the highly compressed condensed monolayer (at $\Pi > 50 \text{ mN/m}$). The $\omega_{(n)}$ value is seen to slightly decrease with the increase of the temperature. At the same time, for the gaseous monolayer the ω value does not depend on the temperature and is equal to 0.44–0.45 nm^2 . The slight decrease of the cohesion constant B (B accounts for the monolayer nonideality) with the increase of the temperature can be ascribed to the weakening of intermolecular interactions, which is usually due to the thermal

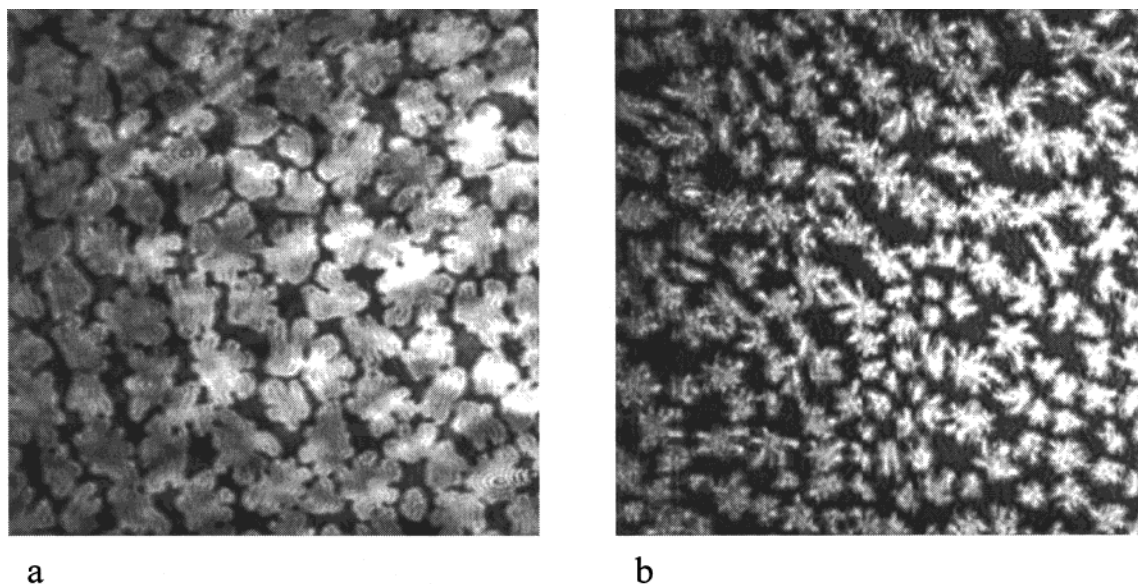


Figure 5. BAM images of fingered condensed phase domains of DPPG monolayers obtained at different compression rates. $T = 34\text{ }^{\circ}\text{C}$. (a) rate = $3.7 \times 10^{-3}\text{ s}^{-1}$, (b) rate = $2.2 \times 10^{-3}\text{ s}^{-1}$.

TABLE 1: Parameters of Π -A Isotherms of DPPG Monolayers and Thermodynamic Characteristics of the Main Phase Transition

temp, $^{\circ}\text{C}$	22	26	30	34
B , mN/m	8.9	7.2	6.5	6.5
ω , nm^2	0.453	0.450	0.445	0.445
$\omega_{(n)}$, nm^2	0.393	0.379	0.360	0.358
A_c , nm^2	1.00	0.90	0.80	0.73
ΔG° , kJ/mol	-2.29	-2.15	-2.00	-1.81
ΔH° , kJ/mol	-14.1	-14.1	-14.1	-14.1
$T\Delta S^{\circ}$, kJ/mol	-11.8	-12.0	-12.1	-12.3

motion. Finally, the critical area A_c at which the aggregation commences is seen to decrease with increasing temperature.

The equation of state described by eqs 1–4 and valid for bimodal distribution between large aggregates and monomers was derived for nondissociated molecules. However, DPPG is an ionic phospholipid and can form charged monolayers on an aqueous subphase. On the other hand, it has been discussed¹⁸ that immediately after spreading on pure aqueous subphase, the DPPG monolayer is hydrolyzed into the respective acid (DPPGH). Therefore, the question arises whether at least partial dissociation of the DPPG monolayers has to be considered or can it be neglected due to the hydrolysis. Dissociation of the monolayer material can lead to phenomena, such as an increase of the area per molecule in the gaseous monolayer or an increase of the surface pressure caused by the increase in the number of kinetic entities, which affect the behavior of the Π -A isotherm in the two-phase coexistence region.

The dissociation of one molecule results in the formation of a pair of ions. Considering the dissociation degree in the generalized Volmer's equation^{19,21}

$$\Pi = RT \frac{\sum_i \Gamma_i}{1 - \sum_i \Gamma_i \omega_i} - B \quad (6)$$

eqs 1 and 5 can be rewritten as²⁴

$$\Pi = \frac{RT(1 + \alpha)(A/A_{c\Pi})\beta}{A - \omega[1 + \epsilon((A/A_{c\Pi})\beta - 1)]} - B \quad (7)$$

$$\Pi = \frac{(1 + \alpha)RT}{A - \omega} - B \quad (8)$$

where Γ_i is the adsorption (surface concentration) value of the i th component or the i th state and α the dissociation degree of the monolayer substance.

Different ionization degrees, $0 \leq \alpha \leq 1$ were tested using eqs 7 and 8. Whereas the agreement between experiment and theory is only little affected for $\alpha \leq 0.1$, it is explicitly worsened for $\alpha \geq 0.1$. Consequently, the effect of dissociation can be largely neglected in DPPG monolayers at medium pH of the aqueous subphase.

For comparison, Figure 6 illustrates the experimental isotherm and a set of theoretical Π -A isotherms for the DPPC phospholipid, which differs from the DPPG molecule in that DPPC contains additionally a positively charged group (N^+) so that the DPPC molecule can be considered to be neutral. For this phospholipid the theory expressed by eqs 1–4 exhibits also good agreement with the experiment. The $\omega_{(n)}$ values for DPPC at 22° exceed those characteristic for DPPG by 0.02 nm^2 at the same temperature. However, the A_c values for DPPC are significantly lower than those for DPPG at the same temperature. The ordering of the DPPC monolayers well studied in the literature^{1–5} is different than that of the DPPG monolayers. The equilibrium domains of DPPC are triskelions with helical changes of the molecule orientation in the three arms.^{3,4} The molecules exhibit an oblique lattice structure with extremely large tilt angles of the chains from vertical, even at highest surface pressure.^{5,25}

Now the thermodynamic results should be discussed in more details, as the temperature dependencies of $\omega_{(n)}$ and A can be used to calculate the standard thermodynamic characteristics of the 2D condensation. In the framework of the quasichemical model of 2D aggregation,²⁰ the two-dimensional aggregation constant $K_n = \exp[(n\mu_1^0 - \mu_n^0)/RT]$ is proportional to the area A_c at which the aggregation commences. Here the chemical potential of the aggregates μ_n and monomers μ_1 is related to each other by the equation $\mu_n = n\mu_1$, where n is aggregation number. A_c is raised to the $(n - 1)$ th power. The expression for A_c in the framework of the phase model of 2D aggregation is even more simple. This model assumes that the chemical potential of the monomers which exist outside of the aggregates,

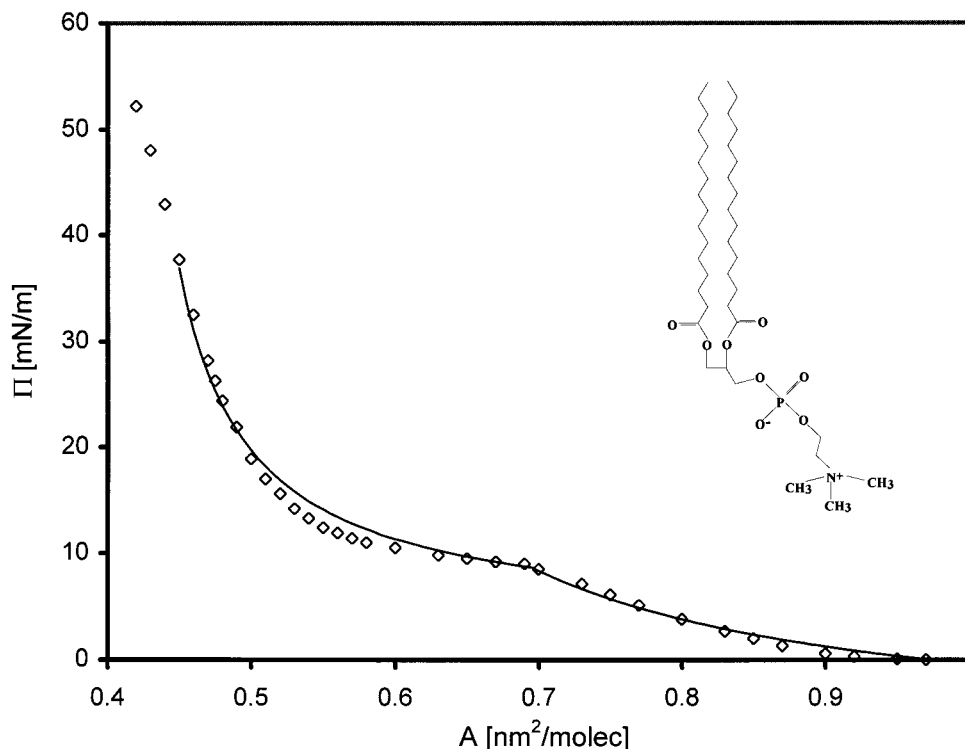


Figure 6. Experimental and theoretical Π - A isotherms for DPPC at temperature 22°. The values of the parameters in eq 1-4 are: $B = 7.8$ mN/m, $\omega = 0.445$ nm², $\omega_{(n)} = 0.414$ nm², $A_c = 0.695$ nm².

μ_1 , is equal to that characteristic for the molecules comprised to the aggregates, $\mu_{1(n)}$, that is, $\mu_1 = \mu_{1(n)}$. The chemical potentials of the amphiphile in the monolayer can be expressed as¹⁹

$$\mu_i = \mu_i^0 + RT \ln f_i x_i - \gamma \omega_i \quad (9)$$

where μ_i^0 is the standard chemical potential dependent on temperature and pressure, f_i the activity coefficient, γ the surface tension, and x_i the molar fraction at interface. In fact, it follows from the condition $\mu_1 = \mu_{1(n)}$ that the surface molar fraction (or surface concentration Γ_1) of the free monomers in the 2D phase transition region is constant: $\Gamma_1 = \Gamma_c = \text{const}$. A similar result follows also from the quasi-chemical model,²⁰ provided large aggregates ($n > 1000$) are formed. Therefore, with $\Gamma_c = 1/A_c$, and assuming that the molecular area of monomers within the aggregates is equal to that outside the aggregates, $\omega = \omega_{(n)}$ (this assumption, although approximate, does not introduce any major bias into the calculations), eq 10 is obtained from eq 9

$$A_c = \omega_{(n)} \exp\left(\frac{\mu_1^0 - \mu_{1(n)}^0}{RT}\right) \quad (10)$$

and, as $A_c > \omega_{(n)}$, the exponent of $(\mu_1^0 - \mu_{1(n)}^0)/RT$ in the right-hand side of eq 10 exceeds 1. This means that the standard chemical potential of free monomers exceeds that within the aggregates, and therefore the difference $\mu_1^0 - \mu_{1(n)}^0$ is always positive, so that the aggregation process, and thus the condensation, is possible. It can be seen that the A_c value decreases due to the decrease of the difference $\mu_1^0 - \mu_{1(n)}^0$ with increasing temperature. As the value of the standard chemical potential of unbounded monomers μ_1^0 is subject only to slight variations with the temperature, this effect can be ascribed to the fact that the standard chemical potential of the aggregated molecules, $\mu_{1(n)}^0$, increases with increasing temperature.

The difference $\mu_{1(n)}^0 - \mu_1^0$ is (by definition) the value of the standard free energy of aggregation ΔG° , calculated per one mole of the monomers. Therefore,

$$\Delta G^\circ = RT \ln(\omega_{(n)}/A_c) \quad (11)$$

Equation 11 is similar to the expression for the standard free energy of the micelle formation for surfactants within the bulk solution²⁶⁻³²

$$\Delta G_m^\circ = RT \ln x_{\text{cmc}} = RT \ln(c_{\text{cmc}}\rho) \quad (12)$$

where ρ is the ratio of the solvent (water) density to its molecular weight, $\rho \approx 1000/18 = 55.6$ [mol H₂O/l], and c_{cmc} is the CMC value expressed in mol/L. In contrast to eq 11, where $\omega_{(n)}/A_c$ denotes the critical molar portion of the amphiphile within the monolayer, eq 12 depends on the critical molar portion of the surfactant within the bulk solution.

The values of standard free energy of aggregation ΔG° for DPPG, calculated from eq 12 at different temperatures, are also presented in Table 1. The ΔG° values are relatively small and negative, and the absolute values decrease with increasing temperature. At the same time, the value of standard free energy characteristic for the micelle formation process is significantly lower (-20 to -40 kJ/mol).²⁶⁻³² This is quite understandable because, in contrast to the aggregation within the monolayer, the micelle formation takes place at very low molar portions of the surfactant within the solution.

The results obtained for ΔG° at various temperatures were used to estimate the values of standard enthalpy (ΔH°) and standard entropy of aggregation (ΔS°)

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (13)$$

$$-\frac{\Delta H^\circ}{T^2} = \frac{\partial}{\partial T} \left(\frac{\Delta G^\circ}{T} \right) \quad (14)$$

TABLE 2: Parameters of Π -A Isotherms of THHGPA Monolayers and Thermodynamic Characteristics of the Main Phase Transition

temp, °C	16	20	26	30
$\omega_{(n)}$, nm ²	0.572	0.552	0.556	0.558
A_c , nm ²	1.13	0.99	0.91	0.88
ΔG° , kJ/mol	-1.64	-1.42	-1.22	-1.15
ΔH° , kJ/mol	-11.7	-11.7	-11.7	-11.7
$T\Delta S^\circ$, kJ/mol	-10.1	-10.3	-10.5	-10.6

The standard entropy of aggregation was calculated using the Gibbs-Helmholtz equation (eq 14), where the ΔG° values were used for two different temperatures among those four values listed in Table 1, so that six ΔH° values were calculated. The average value of $\Delta H^\circ = -14.1 \pm 1.4$ kJ/mol obtained, together with the values of the entropy component of the free aggregation energy $T\Delta S^\circ$, are shown in Table 1. It is seen that the standard entropy of the system decreases significantly during the aggregation in the monolayer. The comparison with the model lipid DPPC is of interest. The value of the standard free energy of aggregation for DPPG (-2.29 kJ/mol) is lower than that of DPPC (-1.27 kJ/mol) according to the differences in the A_c value, which is lower for DPPC than for DPPG.

The $T\Delta S^\circ$ values for the processes of surfactant micelle formation in the aqueous bulk phase are significantly different from those characteristic for the aggregation processes within the monolayer. The values of standard enthalpy for the micelle formation are usually close to zero (-10 to 10 kJ/mol). Consequently, the standard entropy values of the micelle formation are very high as the ΔG° values are very low.²⁸⁻³² This nontrivial effect may be traced back to changes in the water structure, mainly caused by the micelle formation in the aqueous bulk solution. The increase of ΔS° due to micelle formation can be ascribed principally to the disordering of the water structure in the bulk solution.^{33,34} In aqueous solution, single surfactant molecules are surrounded by an ordered water shell which is destroyed by micelle formation. Correspondingly, the entropy of the system increases because the effect of disordering of the water structure overcompensates the entropy decrease in the system due to the aggregation of the surfactant molecules to ordered micellar structures.

Unlike the micelle formation, within the Langmuir monolayer the aggregation of the amphiphilic molecules to highly ordered condensed phases results only in an entropy decrease of the system due to the ordering of the amphiphile molecules. In the nonaggregated fluid-like phase, the number of possible states of molecules decreases simultaneously. That means an entropy increase which compensates this effect does not take place. As the standard free energy of aggregation is small, the decrease of the standard entropy almost compensates the significant decrease of the standard enthalpy of aggregation caused by the interaction between the aggregating molecules. It can be guessed that the $T\Delta S^\circ$ value represents a quantity for characterizing the degree of ordering, i.e., the crystallinity within the condensed phase domains.

Similar features are also characteristic for the 2D main phase transition of Langmuir monolayers of other amphiphiles or phospholipids which form condensed phase domains in the two-phase coexistence region. Even the thermodynamic characteristics of the main phase transition in the monolayers of very special triple-chain phospholipids, such as rac-1-(2-tetradecyl-hexadecanoyl)-2-hexadecylglycerophospho-ethanolamine (THHGPEA) recently studied,²¹ can be interpreted in the same way. Table 2 summarizes some parameters of the Π -A isotherms of THHGPEA and the thermodynamic characteristics of aggregation calculated from these parameters. This system also

provides clearly negative values of standard entropy and enthalpy, which almost compensate each other because the values of the standard free energy of aggregation are low.

Conclusions

The ionic phospholipid DPPG forms stable Langmuir monolayers which provide reliable Π -A isotherms over the accessible temperature range. Consequently, the area values of the compressed monolayers approximate to the cross-section areas of the molecules. The thermodynamic and textural characterization is based on the Π -A isotherms at different temperatures for $25^\circ\text{C} \leq T \leq 34^\circ\text{C}$. In this temperature range, the surface pressure of the main phase transition point depends linearly on the temperature. The formation of condensed phase domains, observed in the two-phase coexistence region is in complete agreement with the thermodynamic phase transition. Equilibrium domains which are compact, but never really round, are formed in the two-phase coexistence region. These domains differ from each other in the azimuthal tilt but they have no inner texture. At increasing compression rate, fingering or fractal-like domain shapes indicate nonequilibrium states which can relax into equilibrium shapes.

The experimental Π -A isotherms can be well described over the entire region of the gaseous and condensed state by an equation of state valid for a bimodal distribution of large aggregates and monomers. The equation of state derived recently is based on the generalized Volmer's equation and the quasi-chemical equilibrium model of 2D aggregation. It is interesting to note that a possible dissociation effect on the monolayer properties of the ionic DPPG can be largely ignored, as concluded from the application of the equation of state extended to the dissociation degree of ionic monolayer material. Comparison with the monolayer properties of model phospholipid DPPC reveals differences, particularly demonstrating the better textural ordering of the DPPC monolayers.

Analysis of the standard thermodynamic characteristics for the 2D aggregation of Langmuir monolayers indicates nontrivial differences to those for aggregation processes in the bulk phase, e.g., for the micelle formation. In Langmuir monolayers, the aggregation of the lipid or other amphiphilic molecules to a highly ordered condensed phase results in an entropy decrease of the system, not compensated by an entropy increase of an other process. This suggests that the $T\Delta S^\circ$ value represents a quantity characterizing the degree of ordering induced by the phase transition.

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References and Notes

- (1) Moy, V. T.; Keller, D. J.; Gaub, H. E.; McConnell, H. M. *J. Phys. Chem.* **1986**, *90*, 3198.
- (2) Moy, V. T.; Keller, D. J.; McConnell, H. M. *J. Phys. Chem.* **1988**, *92*, 5233.
- (3) Weidemann, G.; Vollhardt, D. *Colloids Surf.* **1995**, *100*, 187.
- (4) Weidemann, G.; Vollhardt, D. *Biophys. J.* **1996**, *70*, 2758.
- (5) Brezesinski, G.; Dietrich, A.; Struth, B.; Böhm, C.; Bouwman, W. G.; Kjaer, K.; Möhwald, H. *Chem. Phys. Lipids* **1995**, *76*, 145.
- (6) Mestras, C.; Ortiz, A.; Haro, I.; Reig, F.; Alsina, M. *Langmuir* **1997**, *13*, 5669.
- (7) Mota, F. M.; Busquets, M. A.; Reig, F.; Alsina, M. A.; Haro, I. *J. Colloid Interface Sci.* **1997**, *188*, 81.
- (8) Perezgil, J.; Casals, C.; Marsh, D. *Biochemistry* **1995**, *34*, 3964.
- (9) Williams, A. D.; Wilkin, J. M.; Dluhy, R. A. *Colloid Surf. A* **1995**, *102*, 231.
- (10) Nag, K.; Perezgil, J.; Cruz, A.; Keough, K. M. W. *Biophys. J.* **1996**, *71*, 246.

- (11) Taneva, S. G.; Keough, K. M. W. *Biochim. Biophys. Acta* **1995**, 1236, 185.
- (12) Vonnahmen, A.; Schenk, M.; Sieber, M.; Amrein, M. *Biophys. J.* **1997**, 72, 463.
- (13) Amrein, M.; Vonnahmen, A.; Sieber, M. *Eur. Biophys. J.* **1997**, 26, 349.
- (14) Koppenol, S.; Tsao, F. H. C.; Yu, H.; Zograf, G. *Biochim. Biophys. Acta* **1998**, 1369, 221.
- (15) Möhwald, H. *Rep. Prog. Phys.* **1993**, 56, 653.
- (16) Vollhardt, D. *Adv. Colloid Interface Sci.* **1996**, 64, 143.
- (17) El Mashak, E. M.; Tocanne, J.-F. *Biochim. Biophys. Acta* **1980**, 596, 165.
- (18) Grigoriev, D.; Krustev, R.; Miller, R.; Pison, U. *J. Phys. Chem. B* **1999**, 103, 1013.
- (19) Fainerman, V. B.; Lucassen-Reynders, E. H.; Miller, R. *Colloids Surf. A* **1998**, 143, 141.
- (20) Fainerman, V. B.; Vollhardt, D.; Melzer, V. *J. Phys. Chem.* **1996**, 100, 15478.
- (21) Fainerman, V. B.; Vollhardt, D. *J. Phys. Chem. B* **1999**, 103, 145.
- (22) Volmer, M. Z. *Phys. Chem. (Leipzig)* **1925**, 115, 253.
- (23) Brezesinski, G., unpublished results.
- (24) Fainerman, V. B.; Vollhardt, D.; Johann, R. *Langmuir*, submitted.
- (25) Dahmen-Levison, U.; Brezesinski, G.; Möhwald, H. *Thin Solid Films* **1998**, 327–329, 616.
- (26) Katz, J. L. *J. Colloid Interface Sci.* **1976**, 56, 179.
- (27) Kishimoto, N.; Sumida, K. *Chem. Pharm. Bull.* **1976**, 24, 1226.
- (28) Rosen, M. J. *Surfactants and Interfacial Phenomena*; John Wiley & Sons: New York, 1978.
- (29) Rosen, M. J. In *Solution Chemistry of Surfactants*; Mittal, K. L., Ed.; Plenum: New York, 1979; Vol.1, p 45.
- (30) Rosen, M. J.; Cohen, A. W.; Dahanayake, M.; Hua, X.-Y. *J. Phys. Chem.* **1982**, 86, 541.
- (31) Nakagaki, M.; Yamamoto, M. *Bull. Chem. Soc. Jpn.* **1977**, 50, 873.
- (32) Haque, M. E.; Das, A. R.; Moulik, S. P. *J. Phys. Chem.* **1995**, 99, 14032.
- (33) Markina, Z. N.; Zadymova, N. M.; Bovkun, O. P. *Colloids Surf. A* **1987**, 22, 9.
- (34) Fainerman, V. B. *Colloids Surf. A* **1991**, 57, 249.