

# Wettability and Topography of Phospholipid DPPC Multilayers Deposited by Spin-Coating on Glass, Silicon, and Mica Slides

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The surface free energy of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) layers deposited on glass, silicon, or mica by the spin-coating method was estimated. For this purpose, the advancing and receding contact angles of water, formamide, and diiodomethane were measured, and then two concepts of the interfacial interactions were applied. In the contact angle hysteresis approach, the apparent total surface free energy is calculated from the advancing and receding contact angles of the probe liquids, and in the Lifshitz–van der Waals/acid–base approach, the total surface free energy is calculated from previously determined components of the energy, that is, the apolar Lifshitz–van der Waals and the polar electron-donor and electron-acceptor, which are calculated from the advancing contact angles of the probe liquids alone. Comparison of the results obtained using these two approaches provided more information about changes in the hydrophobic/hydrophilic character of the DPPC layers and, simultaneously, a verification of the approaches. Moreover, the roughness and topography of the investigated layers were also examined by atomic force microscopy measurements. The hydrophilic character of the DPPC layers decreased if up to 0.5 mg of DPPC/mL was used to deposit on the substrates by the spin-coating method. Then it increased and leveled off if up to 2–2.5 mg of DPPC/mL was used. The changes in the energy were correlated with the changes in topography of the surfaces.

## 1. Introduction

Phospholipids are major components of biological membranes. Therefore, they are widely used materials in preparing solid-supported lipid layers as biomimetic systems. Recently, the films of biomolecules have drawn much attention because of their potential applications as biological sensors,<sup>1–3</sup> matrices for enzymes, and drug delivery.<sup>4,5</sup>

There are fundamentally two methods of formation for solid-supported films: the transfer of monolayers from the air–water interface onto a solid support (Langmuir–Blodgett or Langmuir–Schaefer techniques)<sup>6–10</sup> or the deposition (adsorption) of the films from an organic solvent onto solid substrates followed by evaporation of the solvent (e.g., spreading from organic solution<sup>11</sup> or the spin-coating method).<sup>12</sup> The lipid film structure and properties obtained by means of this latter method depends on numerous factors, such as the lipid composition, its concentration, the kind of the solvent, pH, the presence of ions, as well as the kind and roughness of the solid substrate, the spinning frequency of the process, temperature, and pressure.<sup>13</sup> Moreover, the solvent

nature is responsible for its evaporation rate.<sup>14</sup> The spin-coating method is mostly applied for the deposition of relatively thin layers (2–30 layers).<sup>12,15</sup> It allows for fast preparation of the layers with the help of inexpensive equipment. Cohen Simonsen and Bagatolli<sup>16</sup> stated that “*spin-coating is common technique for application of thin, uniform coatings to surfaces*”. To successfully use this technique, the solvent has to wet the solid support.

Among the different techniques that are used to characterize the structure, composition, and mechanical properties of biological materials deposited on a solid support, atomic force microscopy (AFM) is very often used, and can help determine the layer homogeneity and the presence of defects.<sup>17</sup> On the other hand, knowledge of whether the layer surface is wetted by liquids may be of great importance for many practical purposes. The surface free energy of a solid is usually determined by measurements of the contact angles appearing at the three-phase solid/liquid drop/air contact line.

Although numerous papers dealing with lipid layers have been published, to our knowledge investigations of the surface free energy of layers deposited on various solid supports have not yet been studied, except for our paper published previously, where 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) layers were deposited from a solution by its spreading on glass, mica, or poly(methyl methacrylate) (PMMA) surfaces and then the solvent was evaporated.<sup>18</sup> Taking the geometrical surface of the solid substrate and the amount of DPPC in the poured solution, the statistical number of DPPC monolayers has been calculated. In this way, even up to 10 statistical monolayers have been deposited by pouring the solution successively on the dried layers. From AFM analysis, we concluded that, in most cases, the layers

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were probably not homogeneous. The greatest changes (decrease) in the apparent surface free energy of the DPPC layers appeared for the first three statistical monolayers, especially those deposited on glass and mica.

In this paper, we investigated surface free energy and the components of DPPC layers deposited by the spin-coating technique on glass, silicon, and mica slides to compare these results with those published in our previous paper.<sup>18</sup> We wish to learn whether the surface energy changes of such deposited layers correlate somehow with their topography, which was investigated by AFM. The surface free energy of the layers was calculated from the advancing and receding contact angles of probe liquids applying van Oss et al.'s Lifshitz–van der Waals/acid–base (LWAB) approach<sup>19–21</sup> and the contact angle hysteresis (CAH)<sup>22–25</sup> approach.

## 2. Experimental

**2.1. Materials.** 1,2-Dihexadecanoyl-*sn*-glycero-3-phosphocholine (DPPC, semisynthetic, ~99%) was purchased from Sigma and used as received after dissolving in 2,2,2-trifluoroethanol (TFE) (Aldrich). The three probe liquids used for contact angle measurements were water (from a Milli-Q system, 18.2 MΩ cm), formamide (Belgium, > 99%), and diiodomethane (POCh Gliwice, p.a.). These three probe liquids (one apolar and two polar) for contact angle measurements are commonly used to obtain the least error in determination of the surface free energy components.<sup>19–21</sup>

The solid supports for the phospholipid layers were microscope glass slides (Comex, Wrocław, Poland), silicon wafers (ON Semiconductor, Czech Republic), and muscovite mica plates (Continental Trade, Warsaw).

**2.2. Slide and DPPC Layer Preparation.** The DPPC layers were deposited on slides (10 × 26 mm<sup>2</sup>), which were cut from commercial glass microscope covers or a bigger slab of silicon or mica. Then the glass and silicon slides were carefully cleaned in an ultrasonic methanol bath for 10 min, rinsed thoroughly with Milli-Q water, and placed into an ultrasonic bath in Milli-Q water two times for 15 min. The mica slides were only rinsed with methanol and water. Next, all the slides were dried in a desiccator at 100 °C for 30 min. Then, to enhance wetting properties by hydrophilic TFE solvent, the glass slides were kept in a 5 M KOH solution for about 1 min, while the silicon ones were placed in a saturated solution of KOH in ethanol, also for 1 min. After that, the slides were extensively rinsed with Milli-Q water and again placed into an ultrasonic bath in Milli-Q water two times for 15 min, then dried in a desiccator at 100 °C for 30 min. Finally, all the slides were stored in a desiccator with a molecular sieve at room temperature until their use.

The phospholipid layers were obtained using spin-coater model SCI-30 (L.O.T. – Oriel GmbH & Co. KG, Germany). First, 0.1 mL of DPPC solution in TFE was pipetted from a microsyringe onto the surface of previously cleaned substrates. The concentrations of DPPC ranged from 0.25 to 3 mg/mL. After spreading the lipid solution over the surface and wetting the whole substrate surface, the sample was rotated at a speed of 50 rps for 30 s for the solvent to evaporate completely. During the rotation, some volume of the lipid solution could be ejected from the surface. However, as a result, a thin film of the lipid adhered to the surface. After the coating, each of the samples were stored under a vacuum for at least 6 h in order to remove any traces of the solvent and were then kept in a desiccator with a molecular sieve in a fridge until they were used in the measurement. Statistically, if a whole volume of 0.1 mL of the 0.25

mg/mL DPPC solution was spread over and allowed to rest on a 10 × 26 mm<sup>2</sup> slide, it would cover it with 45 DPPC monolayers, assuming vertical orientation of the molecules (0.57 nm<sup>2</sup> the cross-section/molecule). The layers were prepared at room temperature (20 ± 1 °C).

**2.3. Contact Angle Measurements.** The contact angles were measured with the help of a GBX contact angle meter (France) equipped with a video camera system. The advancing contact angles of the probe liquids (water, formamide, and diiodomethane) were measured after settling 3 μL droplets on the surface. Then a 1 μL volume of the liquid was sucked into the syringe from the droplet, and the receding contact angle was read out. All contact angles were measured at room temperature (20 ± 1 °C) under nitrogen atmosphere present in the closed measuring cell to avoid any water vapor adsorption onto the DPPC layer. The readings were taken on the left and right sides of the droplet's profile for all three probe liquids. The contact angles were also measured on the bare surfaces of the substrate slides. Three replicated series of experiments of the contact angle measurements were carried out on each substrate. In each series and for each liquid, the contact angles were measured for at least four droplets. Thus, for the energy determination, the arithmetic mean value of the advancing or receding contact angle of a particular liquid was calculated from 24 readings. Generally, the reproducibility of the contact angle measurements was within two degrees.

**2.4. AFM Investigations.** To examine the topography of the bare support surfaces and those with deposited DPPC films, the slides were cut into small pieces (10 × 10 mm<sup>2</sup>) suitable for the AFM investigations. The AFM measurements were performed with a Nanoscope III (Veeco, USA) with a standard silicon tip, in which contact or tapping mode can be applied. Other parameters of the instrument are as follows: nominal spring constant (*I*) 20–100 N/m, resonant frequency 200–400 kHz, nominal tip radius of curvature 5–10 nm. All the images were obtained at room temperature in open air. The roughness of the surfaces and distribution of heights (topography) were determined by means of WSxM 4.0, Develop 8.0 scanning probe microscope software.<sup>26</sup>

**2.5. Determination of the DPPC Layer Surface Free Energy.** This was determined in the same manner described in our earlier paper.<sup>18</sup> Two approaches to the interfacial interactions based on advancing or advancing and receding contact angles of the probe liquids (e.g., water, formamide, and diiodomethane) were applied. The details can be found elsewhere, and here only the principles will be recalled.

Van Oss et al.<sup>19–21</sup> introduced the concept of surface free energy as a sum of apolar interactions, called Lifshitz–van der Waals  $\gamma_s^{LW}$  and polar Lewis acid–base interactions  $\gamma_s^{AB}$  (LWAB approach):

$$\gamma_s = \gamma_s^{LW} + \gamma_s^{AB} = \gamma_s^{LW} + 2(\gamma_s^+ \gamma_s^-)^{1/2} \quad (1)$$

where  $\gamma_s^-$  is the electron-donor, and  $\gamma_s^+$  is the electron-acceptor components of the energy. Then, using this approach, the work of adhesion  $W_A$  of the solid/liquid can be expressed:<sup>19–22</sup>

$$W_A = \gamma_L (1 + \cos \theta) = 2(\gamma_s^{LW} \gamma_L^{LW})^{1/2} + 2(\gamma_s^+ \gamma_L^-)^{1/2} + 2(\gamma_s^- \gamma_L^+)^{1/2} \quad (2)$$

where the subscripts “S” and “L” mean solid and liquid, respectively.

Accordingly, measuring the advancing contact angles of three different liquids (one of which can be purely apolar) with known surface tension components and simultaneously solving eq 2 for each, one can calculate the surface free energy components and then the total surface free energy of the solid surface. It should be mentioned that, in this approach, it was assumed that, for water,  $\gamma_w^- = \gamma_w^+ = 25.5 \text{ mJ/m}^2$ , and that, irrespective of the probe liquid used, the interactions originating from the solid side are the same,<sup>19–21</sup> which is of course problematic. This is because, using different triads of the probe liquids and measuring their advancing contact angles, the determined components of the same solid surface free

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**Table 1. Surface Free Energy and Its Components in mJ/m<sup>2</sup>, rms Roughness, and Average Heights (both in nm) of the Bare Substrate Surfaces**

substrate	$\gamma_s^{\text{LW}}$	$\gamma_s^+$	$\gamma_s^-$	$\gamma_s^{\text{AB}}$	$\gamma_s^{\text{tot}}$ (LWAB)	$\gamma_s^{\text{tot}}$ (CAH)	rms	aver. height
glass	38.5 ± 0.5	1.0 ± 0.1	50.5 ± 0.8	14.2 ± 0.4	52.7 ± 0.1	55.9 ± 0.2	0.251	1.13
silicon	41.1 ± 0.3	0.1 ± 0.0	56.2 ± 0.7	4.7 ± 0.7	45.8 ± 0.8	55.6 ± 0.4	0.103	0.45
mica	41.3 ± 0.2	0.4 ± 0.0	55.9 ± 0.3	9.5 ± 0.5	50.8 ± 0.6	56.8 ± 0.3	0.215	0.80

energy differ more or less. Furthermore, the electron-acceptor component is usually small. The authors of this approach maintain that it results from the nature of most solids.<sup>19–21</sup> On the basis of the above assumption for water, that  $\gamma_w^- = \gamma_w^+ = 25.5$  mJ/m<sup>2</sup>, the acid–base components of other probe liquids have been determined. So, it is clear that thus determined solid surface free energies and its components are relative ones. But, their changes in a tested system are informative and helpful for understanding interfacial processes.

In the CAH approach, Chibowski<sup>22–25</sup> proposed an equation relating the total surface free energy of a solid ( $\gamma_s^{\text{tot}}$ ) with the surface tension of the probe liquid ( $\gamma_L$ ) and the hysteresis of the contact angle of this liquid, which is defined as the difference between advancing ( $\theta_a$ ) and receding ( $\theta_r$ ) contact angles:

$$\gamma_s^{\text{tot}} = \frac{\gamma_L(1 + \cos \theta_a)^2}{(2 + \cos \theta_r + \cos \theta_a)} \quad (3)$$

Because if this approach is used, the determined surface free energy depends to some extent on the kind of probe liquid used; therefore it suggests that thus calculated values characterize the apparent surface free energy. It is believed that the differences in the values of the energy as determined from the hysteresis of different probe liquids are due to changing strengths of the solid/liquid interfacial interactions, which reflect in the measured contact angles, for example, of diiodomethane, water, or formamide. If diiodomethane is used as the probe liquid, the interactions are practically of dispersive nature only, but those with water and formamide are of the electron-donor and electron-acceptor too.

Maybe it is worth briefly commenting on the general problem of solid surface free energy determination. Namely, it appears that the averaged values of the energy from the CAH approach agree very well with the mean values from the LWAB approach, especially if several triads of the probe liquids are used for advancing contact angle measurements.<sup>22,23</sup> Therefore, it can be said that both methods are complementary, and because there are no better methods for solid surface free energy determination, comparison of the results obtained by these two can be helpful, for example, for better understanding of the hydrophobic–hydrophilic properties of the lipid membranes.

### 3. Results and Discussion

#### 3.1. Surface Free Energy and Roughness of Bare Substrates.

The physicochemical properties of solid-supported phospholipid layers are important for the characterization and development of novel biomolecular materials (e.g., biosensors). The changes in the surface wettability of DPPC layers deposited on glass, silicon, and mica can be evaluated from their surface free energy determined by means of the two approaches and using the measured contact angles and surface tension as well as the components of the probe liquids.<sup>27</sup>

Table 1 contains values of the total surface free energy of the bare surfaces of the substrates calculated from van Oss et al.'s LWAB approach and the CAH approach. The  $\gamma_s^{\text{tot}}$  values calculated from CAH are arithmetic mean values of the apparent surface free energies determined from the CAH of water, formamide, and diiodomethane. Also given are the experiment errors in the values of free energy and its components calculated respectively from the maximum and minimum contact angles

measured on a given support. The errors indicate that the measured contact angles were well reproducible.

The values calculated from LWAB are a little lower than those from CAH, and they show that silicon possesses the lowest surface free energy and acid–base interaction  $\gamma_s^{\text{AB}}$ , which is the highest for the glass surface. The electron-acceptor parameter  $\gamma_s^+$  of the substrate surfaces (Table 1) is small, which, for most solids, is typical if the LWAB approach is used.<sup>21–23</sup> The apolar interactions  $\gamma_s^{\text{LW}}$  are comparable and are only a bit lower for glass. The  $\gamma_s^{\text{tot}}$  values for glass and mica determined previously from the CAH approach were practically the same (54.3 and 57.2 mJ/m<sup>2</sup>, respectively).<sup>18</sup> From the above values of the energy and its components, one may conclude that properties of the DPPC layers deposited on these substrates may not differ drastically, especially if the topography of the surfaces are found to be similar. The topography was evaluated from AFM images,<sup>26</sup> and the root-mean-square (rms) roughness and average height values for the bare surfaces are collected in Table 1. As can be seen, silicon exhibits the least rms roughness (0.103 nm), with an average height of 0.45 nm, followed by mica (0.215 and 0.80 nm, respectively) and then glass (0.251 and 1.13 nm, respectively). It appears that the sequence of roughness changes in the same way as  $\gamma_s^{\text{AB}}$  and  $\gamma_s^{\text{tot}}$  (LWAB) (Table 1). The results in Table 1 show that all three surfaces are in fact molecularly flat with a very narrow distribution of the heights (not presented here). For example, in the case of silicon, they are mostly between 3 and 6 Å. Thus, from both the surface free energies and the topographies, it may be expected that the energetic properties of DPPC layers deposited on these substrates may be similar indeed. Previously used glass was of “worst surface” quality, because the rms roughness and the average height were 0.946 and 2.61 nm, respectively, while the mica showed similar parameters (0.276 and 0.57 nm, respectively).<sup>18</sup> Because the support surfaces are polar ones, in the first adsorbed layer, the DPPC molecules (whose length is 2.97 nm<sup>28</sup>) should be directed with their polar (ionic) part toward the polar sites present on the surfaces.<sup>29</sup>

**3.2. Surface Free Energy of DPPC Layers.** **3.2.1. The Surface Free Energy Calculated from the LWAB Approach.** The values of total surface free energy calculated from the advancing contact angles of water, formamide, and diiodomethane (LWAB approach) versus the concentrations of DPPC solutions used in the spin-coating are presented in Figure 1. Obviously the amount of DPPC left on the substrate surface after the solvent evaporation is probably less than the amount used, because some volume of the solution could be ejected from the surface by the centrifugal force. It is hardly possible to estimate how much of the phospholipid had adsorbed to the interface. Nevertheless, the AFM images allow conclusions to be made about the structure and thicknesses of the deposited layers.

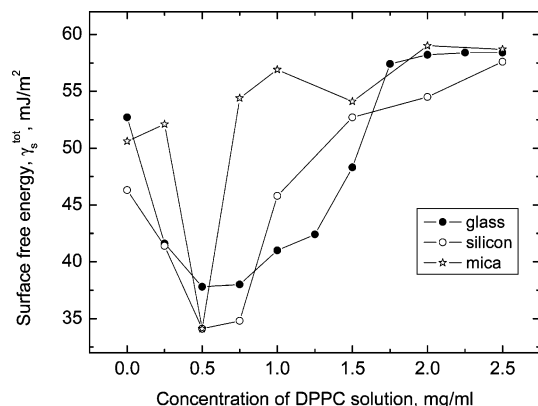
As it was discussed above in the Experimental section, thus calculated values of the energy are both relative and apparent ones. However, their changes are meaningful, and they reflect changes in the intermolecular interactions with the particular probe liquid used for the energy determination. The changes

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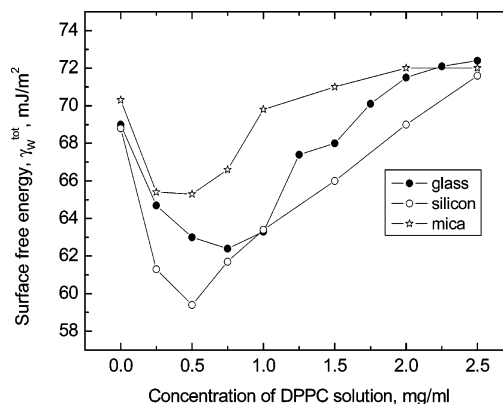




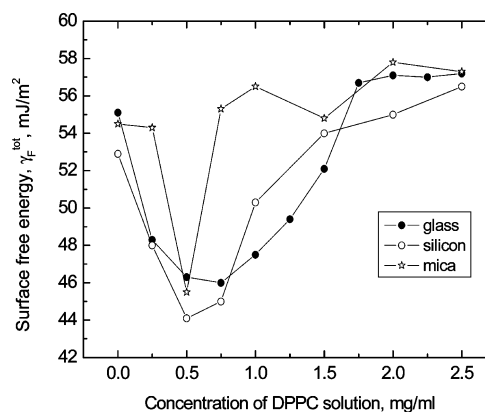
**Figure 1.** Changes in total surface free energy of DPPC layers deposited on glass, silicon, or mica calculated from the LWAB approach versus the concentration of DPPC solution used for the layer deposition by spin-coating method.

may result from the treatment of the layers with water. Keeping in mind the assumptions applied in the approaches, the calculated free energy values characterize the film layer (the film surface)/gas interface, although they were evaluated via the interfacial film surface/probe liquid interactions occurring after the droplet settling. It should be also stressed that, with prolonging the time of contact angle measurements in these systems, some changes in the DPPC layer structure can occur due to contact with water, which may affect the measured contact angle. However, the contact angle measurement was usually completed within 1 min. Therefore, one can expect that the changes, if any, are not extensive ones. Unfortunately, so far, there is no other method for solid surface free energy determination without contact with another condensed phase (a liquid). Nevertheless, the results presented in this paper show that even these apparent values of the energy are helpful, especially in connection with AFM images, in obtaining better characterization of the processes taking place in the DPPC layers.

Focusing now on the free energy changes shown in Figure 1, there is a distinct minimum appearing if 0.5–0.75 mg/mL of DPPC solution was used for the deposition. After reaching this point, the energy values increase upon increasing the surface coverage and level off at 2–2.5 mg/mL. Moreover, at this coverage, the energy is the same regardless of the nature of the substrate, but is greater than those of bare substrates. Further increase in the DPPC concentration did not change the energy (not shown here). As it was expected, the general trends in the energy changes on these three substrates are very similar, but the specificity of the supports is seen too. This is probably due to the differences in the free energy components of the particular solid substrates (Table 1). However, the same surface free energy of DPPC layers deposited from 2.5 mg DPPC/mL on the three different substrates suggests that the values characterize the DPPC molecules themselves. For DPPC layers deposited by spreading and the solution evaporation, which were studied in the previous paper,<sup>18</sup> the energy decrease was observed for the first three statistical monolayers on glass and the first two on mica. Moreover, within experimental error, those minimum values of the energy are comparable with those obtained in this paper (Figure 1). Hence, it may be concluded that the amount of DPPC deposited by spin-coating from a 0.50 mg/mL solution corresponds to about two to three statistical monolayers. The decrease in the surface free energy should result from the reduced polar interactions, as well as the decreased the apolar ones, if the hydrocarbon chains are directed outward from the DPPC film surface. Moreover, on a polar support and if the DPPC monolayer



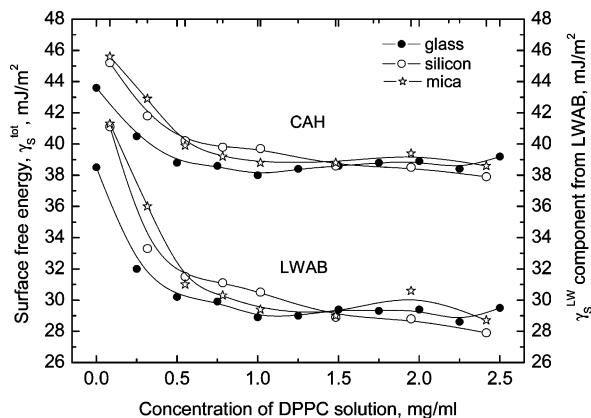
**Figure 2.** Changes in the total apparent surface free energy of DPPC layers deposited on glass, silicon, or mica calculated from water CAH (CAH approach) versus the concentration of DPPC solution used for the layer deposition by spin-coating method.



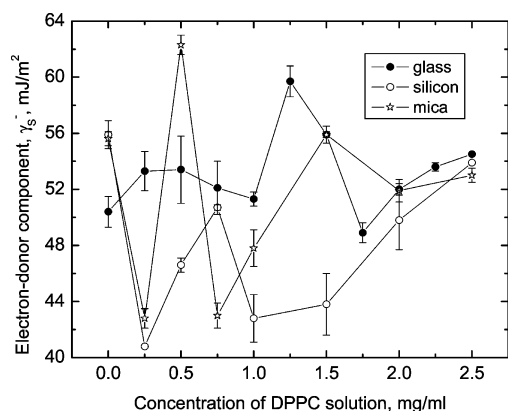
**Figure 3.** Changes in the total apparent surface free energy of DPPC layers deposited on glass, silicon, or mica calculated from formamide CAH (CAH approach) versus the concentration of DPPC solution used for the layer deposition by spin-coating method.

structure is homogeneous, its free energy should be lower than that of the bare substrate because the hydrocarbon tails are directed outward from the surface. But if the monolayer is not uniform, then a higher coverage would be needed to obtain the energy minimum.

**3.2.2. The Surface Free Energy Calculated from the CAH Approach.** The apparent surface free energy of the layers was also calculated from the CAH of water, formamide, and diiodomethane (CAH approach). The results are plotted in Figures 2–4, respectively. In the case of the polar probe liquids (i.e., water and formamide), the energy changes run in a manner similar to that of those from the LWAB approach (Figure 1), especially those calculated from formamide CAH (Figure 3). However, if the energy of the layers is calculated from apolar diiodomethane CAH (Figure 4), it initially decreases up to 0.5 mg DPPC/mL and then is practically constant. Moreover, if more than 1.0 mg/mL DPPC solution is used, the values are similar irrespective of the kind of substrate. This means that these values characterize the London dispersion interactions of DPPC molecules themselves. On the same figure are plotted Lifshitz–van der Waals interactions  $\gamma_s^{LW}$  calculated from the LWAB approach using the advancing contact angles of diiodomethane (eq 2). Generally, these  $\gamma_s^{LW}$  values are lower than the  $\gamma_s^{tot}$  determined from the CAH (eq 3), although, for bare substrate surfaces, the differences are much less. The reason for greater  $\gamma_s^{tot}$  compared to  $\gamma_s^{LW}$  might be that the former results from closer interacting distances.<sup>30</sup> It is because the receding contact angles are also used in the calculations. This issue was discussed more in a previous paper.<sup>18</sup>



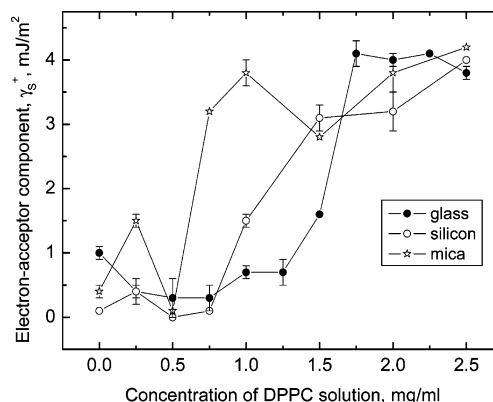
**Figure 4.** Changes in the total apparent surface free energy  $\gamma_s^{\text{tot}}$  of DPPC layers deposited on glass, silicon, and mica calculated from diiodomethane CAH (CAH approach), and the Lifshitz–van der Waals (London dispersion) component  $\gamma_s^{\text{LW}}$  calculated from the advancing contact angles of diiodomethane (LWAB approach).



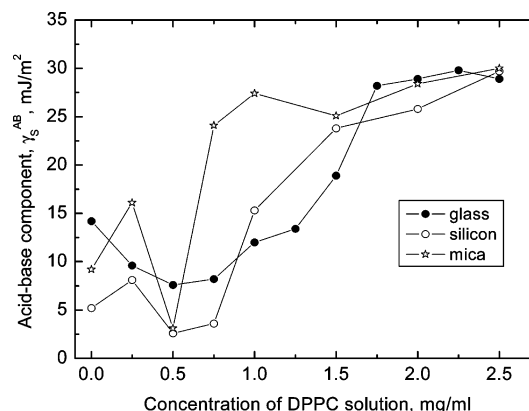
**Figure 5.** The electron-donor  $\gamma_s^{\text{D}}$  component of DPPC layers deposited on glass, silicon, and mica versus the concentration of DPPC solution used for the layer deposition by spin-coating method.

It should be noted that both  $\gamma_s^{\text{LW}}$  and  $\gamma_s^{\text{tot}}$  of the DPPC layers are lower than those of underlying bare substrates. The fact that, for thicker coverages (above 1.0 mg/mL), the values are almost constant (Figure 4), suggests that diiodomethane molecules have similar access to the hydrocarbon chains of DPPC molecules.

**3.2.3. The Electron-Donor and Electron-Acceptor Components of the DPPC Layer Surface Free Energy.** Evaluation of the electron-donor  $\gamma_s^{\text{D}}$  and electron-acceptor  $\gamma_s^{\text{A}}$  interactions should be helpful for better understanding the changes in the total surface free energy of the layers. Figure 5 shows the  $\gamma_s^{\text{D}}$  interactions of the layers calculated from the LWAB approach for the studied substrates, where the standard deviations are also marked by vertical bars. As can be seen, the  $\gamma_s^{\text{D}}$  of DPPC layers deposited on glass does not change much, except for in the concentration range 1.0–2.0 mg/mL, where a maximum of  $\gamma_s^{\text{D}}$  appears if 1.25 mg/mL solution is used. It is characteristic that, regardless of the kind of support, the electron-donor interactions of thick DPPC layers are very similar (ca. 53–54 mJ/m<sup>2</sup>; Figure 5). This value is close to that of a bare glass surface; therefore the changes of this parameter on glass may be the smallest (Figure 5). The electron-acceptor  $\gamma_s^{\text{A}}$  interactions of the layers were also evaluated from LWAB, and the values are presented in Figure 6. Similar to the electron-donor interactions, with increasing thickness of the layers,  $\gamma_s^{\text{A}}$  also tends to the same value (ca. 4 mJ/m<sup>2</sup>)



**Figure 6.** The electron-acceptor  $\gamma_s^{\text{A}}$  component of DPPC layers deposited on glass, silicon, and mica versus the concentration of DPPC solution used for the layer deposition by spin-coating method.



**Figure 7.** The acid–base  $\gamma_s^{\text{AB}}$  component of DPPC layers deposited on glass, silicon, and mica, calculated from the LWAB approach versus the concentration of DPPC solution used for the layer deposition by spin-coating method.

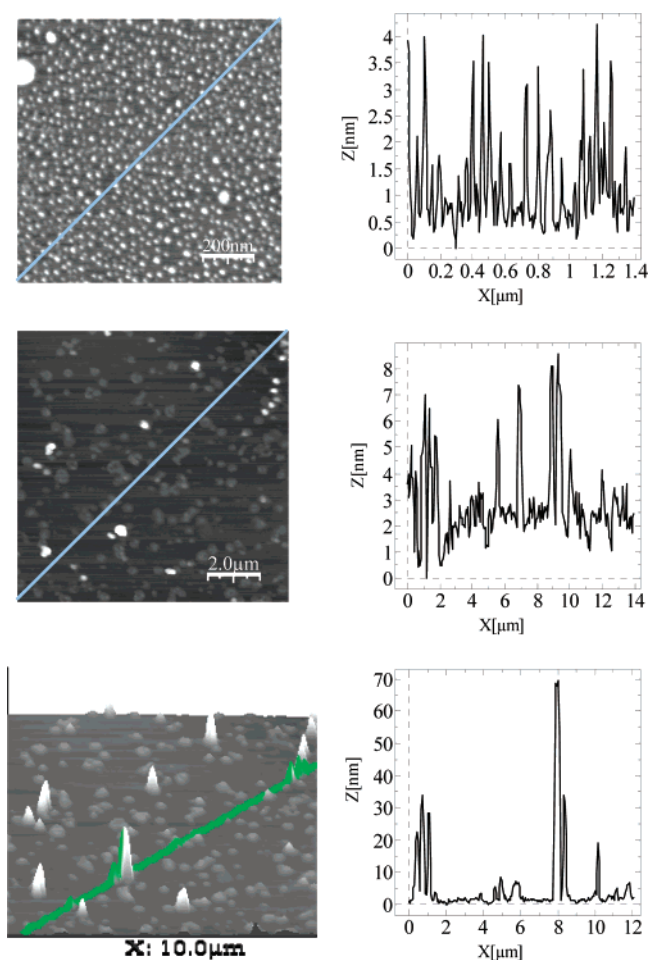
irrespective of the kind of support. These interactions are small and, for thin DPPC layers (up to 0.5 mg/mL), are actually negligible. For the layers on glass, the  $\gamma_s^{\text{A}}$  component first slightly decreases, and, as a result, the acid–base interaction  $\gamma_s^{\text{AB}}$ , which is the geometric mean of  $\gamma_s^{\text{A}}$  and  $\gamma_s^{\text{D}}$  (eq 1), also decreases by ca. 7 mJ/m<sup>2</sup>, which is shown in Figure 7. Because  $\gamma_s^{\text{LW}}$  (London dispersion) interactions also simultaneously decrease by ca. 8 mJ/m<sup>2</sup> (Figure 4), the total surface free energy  $\gamma_s^{\text{tot}}$  decrease of thinner DPPC layers on glass is about 15 mJ/m<sup>2</sup>, as is seen in Figure 1. Thus, the energy decrease is caused by smaller apolar and polar interactions. It worth noting that standard deviations show that the discussed changes in the energy components are statistically significant (Figures 5 and 6).

On the basis of the results plotted in Figures 1, 4, and 5–7, a similar detailed discussion can be conducted for the DPPC layers on silicon and mica. It is worth noting that changes in the  $\gamma_s^{\text{D}}$  of thin DPPC layers (up to 0.75 mg/mL) on mica are the greatest (Figure 5). The value  $\gamma_s^{\text{D}}$  63 mJ/m<sup>2</sup> (a bit greater than that of a bare mica surface, Table 1), like others, was calculated from about 20 contact angles measured in three replicated experiments for each probe liquid. On the other hand, such great  $\gamma_s^{\text{D}}$  has been evaluated only at 0.5 mg DPPC/mL. In a previous paper<sup>18</sup> this component of the DPPC layers on mica was also greater than those on glass and PMMA. Because an increase in the electron-donor  $\gamma_s^{\text{D}}$  is usually accompanied by a decrease in the electron-acceptor  $\gamma_s^{\text{A}}$  interactions, the resulting acid–base  $\gamma_s^{\text{AB}}$  value better reflects changes in the layer polarity. It may be concluded that the changes must be due to DPPC film structure

reorganization with increasing deposited amounts. To find an explanation for the observed changes, more experiments will be conducted next using Langmuir–Blodgett and Langmuir–Schaefer techniques for mono- and bilayer deposition on this solid support.

**3.2.4. General Discussion on the Surface Free Energy of DPPC Layers.** Although, for thin DPPC films, the changes in the interactions on each substrate are specific, the most characteristic feature is that, for thick DPPC layers, if 2.5 mg/mL was used, the energetic properties of the layers are quite similar for both apolar and polar acid–base interactions. Generally, upon increasing the deposited amounts (from more than 0.5 mg/mL solution), the polar character of the DPPC layers deposited on the studied substrates increases (Figure 7). In the case of thinner DPPC layers (up to 0.5 mg/mL), both apolar dispersive and polar acid–base interactions decrease, and then the polar character of the layers increases, while the apolar one is practically the same (Figure 4). It should be mentioned that, when DPPC layers were formed by spreading of the solutions, for two statistical monolayers deposited on glass (mica and PMMA), the  $\gamma_s^{\text{tot}}$  values calculated from the LWAB approach were similar (ca. 42–44 mJ/m<sup>2</sup>).<sup>18</sup> The minimum apparent  $\gamma_s^{\text{tot}}$  obtained in this paper for the layers deposited on all three substrates is 35–38 mJ/m<sup>2</sup> (Figure 1). Comparing previous and present results, one can conclude that the resulting thickness of the layers deposited from 0.5 mg DPPC/mL by the spin-coating technique corresponds to two to three statistical monolayers obtained by pouring DPPC solution at an appropriate concentration.<sup>18</sup> This is now also confirmed by preliminary results obtained for monolayers and bilayers deposited by Langmuir–Blodgett and Langmuir–Schaefer techniques on glass.

**3.3. Topography of the DPPC Layers.** It seemed interesting to us to question whether the surface free energy correlates somehow to the DPPC layer topography. There is vast literature dealing with investigations of supported phospholipid layers on various substrates. The application of very modern techniques (e.g., AFM, frictional force microscopy, or near-field scanning optical microscopy) delivered pictures showing the structure and topography of the deposited layers.<sup>31</sup> However, to compare the free-energy changes and the layer structures, such results should be obtained using samples of the same origin. Therefore we have obtained 1 × 1 μm<sup>2</sup> and 10 × 10 μm<sup>2</sup> AFM images of DPPC layers deposited from 0.5 mg/mL solution on glass, silicon, and mica. They are shown in Figures 8–10, with the surface roughness appearing along the marked lines (from top right to bottom left), and in Table 2 are given the rms roughness and average heights of these layers scanned over 1 μm<sup>2</sup> and 100 μm<sup>2</sup>.<sup>26</sup> Some differences in the layer structures depending on the kind of support are seen in the figures. In all cases, there are some domains present. The most uniform and uniformly distributed DPPC domains are those seen on the 100 μm<sup>2</sup> silicon surface (Figure 8). The AFM image of a DPPC layer on mica shows large islets, some with heights up to 16 nm (Figure 9). A 1 μm<sup>2</sup> AFM image of one of these islets consists of small domains, whose heights are about bilayer thickness (5 nm) (Figure 9). But on the silicon support, apart from the very uniform distribution of (probably) bilayer domains, there are also several visible high peaks (see the three-dimensional (3D) image), whose heights reach even ca. 70 nm (Figure 8). On the glass support (Figure 10), the DPPC domains are also seen, but the images are quite similar for 1 and 100 μm<sup>2</sup>. The large white domains are about 7 nm high, which corresponds to a thickness of about three DPPC monolayers. Cohen Simonsen and Bagatolli<sup>16</sup> using spin-coating technique

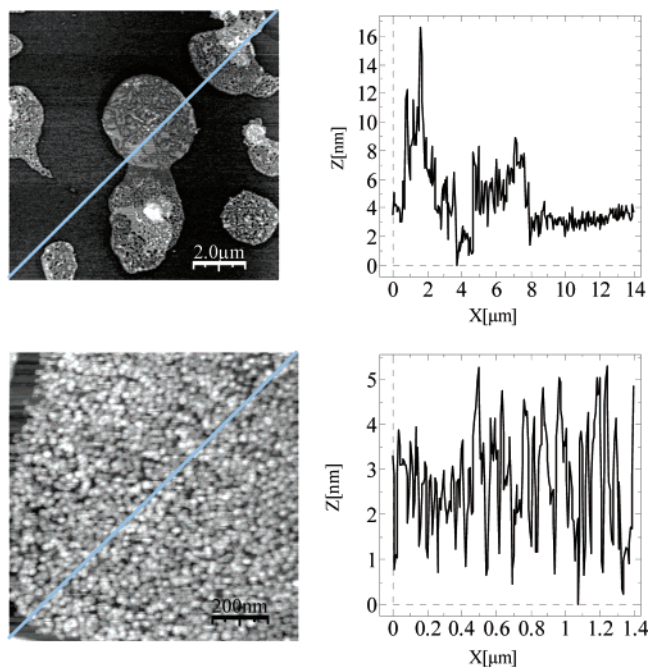


**Figure 8.** Two-dimensional (2D) AFM images of 10 × 10 μm<sup>2</sup> and 1 × 1 μm<sup>2</sup> surfaces of a DPPC/silicon layer obtained from a 0.5 mg/mL solution, the surface roughness along the marked lines (from top right to bottom left), and the surface topography. At the bottom there is a 3D image of the same 10 × 10 μm<sup>2</sup> DPPC/silicon surface and its roughness along the marked line (from top right to left bottom).

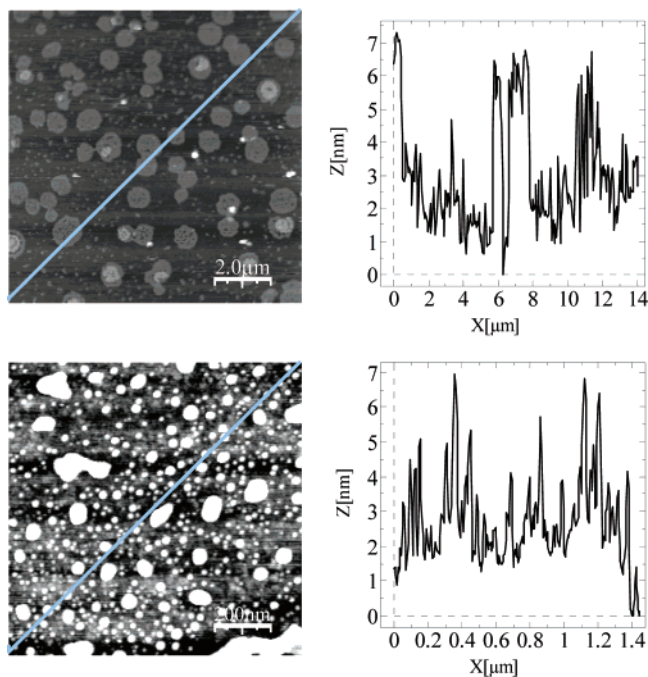
studied the lipid layers deposited on muscovite mica. They used 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and DPPC. From the AFM images, they concluded that, if 15–20 μL of the solution (5 mg of POPC/mL) was spread over an 8 × 8 mm<sup>2</sup> mica sheet using a spin-coater, the surface was completely covered by the lipid, and no holes of the uncovered surface were present. However, if the solution containing 2 mg/mL was spread, uncovered areas appearing as black holes (dark contrasts) were clearly seen on the AFM “phase image”. The authors<sup>16</sup> also concluded that a dry film of the lipid layer on mica was of a monolayer thickness. However, black domains on an AFM “topographical image” are not necessarily due to uncovered patches on the surface.<sup>16</sup> When we transformed the topographical images shown in Figures 8–10 to the phase images (not presented here), no black holes or contrasts were seen. Therefore, it can be concluded that, if 0.5 mg of DPPC/mL solution is used, for none of the substrates used here there are uncovered large patches on their surfaces. The authors<sup>16</sup> also measured the force between the AFM tip and mica or mica–POPC-coated surfaces. They found that, at relative humidity RH = 50–52%, the decrease in the adhesion force relative to the uncovered mica surface amounted to about 40%. From Figure 1, one can evaluate the decrease in the total surface free energy of a DPPC layer on mica (0.5 mg/mL solution, nitrogen atmosphere), which amounts to 33%, which is comparable to the one above. Hollars and Dunn,<sup>31</sup> using confocal microscopy and AFM, found in a DPPC layer

(31) Hollars, C. W.; Dunn, R. C. *Biophys. J.* **1998**, *75*, 342.





**Figure 9.** 2D AFM images of  $1 \times 1 \mu\text{m}^2$  and  $10 \times 10 \mu\text{m}^2$  surfaces of a DPPC layer on mica obtained from a 0.5 mg/mL solution and the surface roughness along the marked lines (from top right to bottom left).



**Figure 10.** 2D AFM images of  $10 \times 10 \mu\text{m}^2$  and  $1 \times 1 \mu\text{m}^2$  surfaces of a DPPC layer on glass obtained from a 0.5 mg/mL solution and the surface roughness along the marked lines (from top right to bottom left).

deposited on mica by the Langmuir–Blodgett technique large circular liquid-condensed (LC) domains surrounded by homogeneous liquid-expanded (LE) regions. The LC domains were 5–8 Å higher than the LE regions. But, on the AFM image, the LE regions appeared to be nonhomogeneous and consisted of small domains. Using near-field fluorescence and topography measurements, the authors pointed out that the domains resulted from the coexistence of the distinct lipid LC and LE phases, and no defects in the DPPC monolayer were observed. They also found that, when the second DPPC monolayer was deposited by

**Table 2.** The rms and Average Heights for DPPC Layers Deposited from 0.5 mg/mL Solutions on Glass, Silicon, and Mica, and from the 2.5 mg/mL Solution on Glass

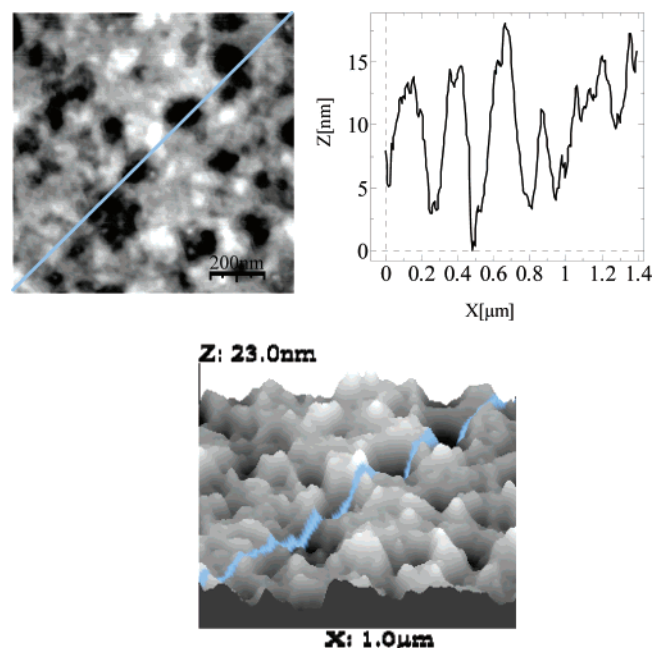
surface	surface size scanned, $\mu\text{m}^2$	rms, nm	average height, nm
glass/DPPC	1	1.51	3.80
	100	2.24	3.50
silicon/DPPC	1	0.89	1.41
	100	4.25	5.23
mica/DPPC	1	1.08	3.41
	100	2.16	4.83
glass/ DPPC, 2.5 mg	1	3.47	12.9

the Langmuir–Schaefer technique on the top of the first one, discrete height changes appeared ( $\sim 8$  Å) in the AFM image, which were about the difference in height between the LE and LC phases. Thus, they observed three height levels, that is, LE on LE, LE on LC (or reverse LC on LE), and LC on LC. Moreover, they also stated that the small domains (mentioned above) were common features regardless of the substrate nature (for example, glass). These authors also found that, in humid conditions, the small DPPC domains aggregated and formed larger domains of like phase. In fact, our results of the free energy changes (Figures 1–4) and the AFM images (Figures 9–11) generally also lead to similar conclusions. In Figure 9 some “aggregates” reach a height up to 16 nm (ca. three bilayers), although their average height is only about a monolayer (4.83 nm, Table 2). Oliynik et al.<sup>32</sup> deduced from AFM images that, by direct fusion of small unilamellar vesicles on mica, a flat gel or fluid layers of DPPC were obtained. It consisted of an  $\sim 5$ –6 nm thick bilayer, and then, on the first bilayer, another bilayer of islets (about 8 nm thick) was formed. Because the thickness of the second bilayer was about 2 nm thicker, they concluded that a water layer was present between the bilayers. However, some black spots that had been seen on the images they recognized as the uncovered mica surface. On the AFM  $10 \times 10 \mu\text{m}^2$  image of the DPPC layer on mica shown in Figure 9 and the roughness profile along the marked line, three distinct levels can be seen, that is, about 2–3, 5–6, and 10–16 nm, so the thicknesses are about that of a monolayer, a bilayer and two to three bilayers, respectively. Summarizing, it can be concluded that the structure and thickness of the phospholipid layers strongly depend not only on the solid support used, but also on the method of their preparation. Therefore, even on the same support, homogeneous or fractured DPPC layers can be obtained.

**3.4. Relation between the DPPC Layer Structure and Its Surface Free Energy.** Leufgen et al.,<sup>33</sup> using time-of-flight secondary ion mass spectrometry (TOF–SIMS), investigated a DPPC layer deposited on a glass slide that had been partially covered with gold. This allowed them to visualize and analyze the structure and composition of the different phases of demixed Langmuir–Blodgett films. Although packing of the molecules was higher in LC than LE phases, the detected amount of protonated DPPC molecules was less. The authors<sup>33</sup> concluded that the reason for this might be the stronger intermolecular interactions in the LC phase. These results shed light on our results of the changes in total surface free energy (Figures 1–3) and on the increase in acid–base interactions (Figure 7) with increasing thickness of the DPPC layer, where more LC phase can be expected. As can be seen in these figures, the total surface free energy is practically the same, irrespective of the kind of support, if the layers were deposited from a solution containing

(32) Oliynik, V.; Kaatz, U.; Heimburg, T. Cornell University arXiv Site. <http://arxiv.org/abs/physics/0609054>. (accessed September 7, 2006).

(33) Leufgen, K. M.; Rulle, H.; Benninghoven, A.; Sieber, M.; Galla, H. J. *Langmuir* **1996**, *12*, 1708.



**Figure 11.** 2D and 3D AFM  $1 \times 1 \mu\text{m}^2$  images of a thick DPPC layer on glass obtained by coating from a 2.5 mg/mL solution. The surface roughness is that along the marked line (from top right to bottom left).

2.5 mg of DPPC/mL. The same is true for the apolar (Figure 4) and acid–base interactions (Figure 7). As an example, an AFM image of such a thick DPPC layer deposited on glass from a 2.5 mg/mL solution is shown in Figure 11 for a  $1 \times 1 \mu\text{m}^2$  surface. The changes in the layer thickness taking place along the marked line (from top right to bottom left) show that the black spots are about a bilayer thick (ca. 5 nm), while the white ones are 12 or 16 nm high (see also the 3D image), which corresponds to one or two bilayers built-up on the first one. As it was discussed above, the black spots on the AFM images (Figures 8–11) are not uncovered patches of the supports, which was concluded from the shapes of the phase images (not presented). Therefore, indeed the kind of underlying support is not important for the outermost layer interactions with the probe liquids if 0.5 mg/mL and more of the DPPC solution is used for spin-coating (Figures 1–4 and 7). From the topography diagram (not shown) for the layer depicted in Figure 11, most of the surface possesses ca. 12–16 nm heights, that is, two to three DPPC bilayers in thickness. However, the rms roughness of this thick layer is only 3.47 nm, but the average height is 12.9 nm (Table 2). The apolar Lifshitz–van der Waals  $\gamma_s^{\text{LW}}$  interaction (from the LWAB approach) of such thick layers is about 28–30 mJ/m<sup>2</sup>, while the total apolar interaction calculated from CAH amounts to 38–39 mJ/m<sup>2</sup> (Figure 4).<sup>25,30</sup> The acid–base contribution to the total surface free energy of these layers amounts to about 30 mJ/m<sup>2</sup> (Figure 7).

**3.5. Wettability of the DPPC Layers.** The above values of surface free energy and its components for a thick layer may be considered as specific ones for the DPPC surface itself. The average advancing contact angle of water on such layers (i.e., deposited on glass, silicon, and mica) equals  $\theta_A = 10.0 \pm 2.6^\circ$ . Hence, the work of spreading  $W_s$ , which is equal to the difference between the work of adhesion ( $W_a = \gamma_l (1 + \cos \theta_A) = 144.5 \text{ mJ/m}^2$ ) and the work of cohesion ( $W_c = 2\gamma_l = 145.6 \text{ mJ/m}^2$ ), equals only  $-1.1 \text{ mJ/m}^2$ . It means that water almost completely

spreads on DPPC surface. This is because of the relatively high electron-donor  $\gamma_s^-$  interactions (ca. 53 mJ/m<sup>2</sup>, Figure 5) and the much weaker, but remarkable, electron-acceptor ones (ca. 3.8 mJ/m<sup>2</sup>, Figure 6). They must originate from the polar (ionic) head of DPPC molecules where negatively charged oxygen and positively charged nitrogen atoms are present, as well as the electron-donor oxygen atoms interacting by hydrogen bonding. On the other hand, calculation of the work of spreading for water on the DPPC layer deposited from a 0.5 mg/mL solution (the minimum in the DPPC layers surface free energy, Figure 1) gives  $W_s$  values equal to  $-15.0$ ,  $-21.9$ , and  $-11.5 \text{ mJ/m}^2$ , on glass, silicon, and mica, respectively. These values show that the least hydrophilic (or the most hydrophobic) is the layer deposited on silicon, which is also the most homogeneous (Figure 8). The increased negative work of the spreading of water at this coverage relative to that on a thick DPPC layer calculated above must result from a higher number of hydrocarbon chains of the molecules exposed outward (Figures 9–11). As was discussed above, in the previous study<sup>18</sup> of DPPC layers deposited by evaporation of the solvent, it was found that a minimum in the total surface free energy occurred for two statistical monolayers on mica and three monolayers on glass. Upon increasing the coverage (up to 7 on mica and up to 10 on glass), the energy increased from 39 to 47 mJ/m<sup>2</sup>, and on glass, within the experimental error ( $\pm 2 \text{ mJ/m}^2$ ), it was the same. The comparison allows concluding that properties of the DPPC layers, as for their surface free energy and structure, at low coverages, depend not only on the kind of support, but also on the method of their preparation, but some common features appear.

#### 4. Conclusions

In the present study, the apparent surface free energy of DPPC layers deposited by spin-coating on solid supports (glass, silicon, and mica) has been evaluated from the measured advancing and receding contact angles of three probe liquids. Two approaches were employed, that is, the CAH approach and the components (i.e., apolar LWAB) approach, for the energy evaluation. Similar trends in the free energy changes were obtained from these two approaches. Although the determined values of the energy are relative and apparent, their changes allow conclusions to be made about the changes in the hydrophobic/hydrophilic character of the surface of DPPC layers. The results show that the energy and surface AFM topographies correlate. The structures of the DPPC layers obtained by spin-coating are generally in agreement with the literature data.

The apolar and polar acid–base components of the film surface free energy change with the amount of deposited DPPC. At low coverages (from a solution below 1.75 mg/mL), the components to some extent depend on the kind of support. However, at higher coverages (from a 2–2.5 mg/mL solution), the components are practically the same, irrespective of the kind of support. Comparison with the results obtained previously by spreading and evaporation of the solvent shows that, although the properties of DPPC layers depend on the method of their preparation, they also show some common features, such as the minimum in the surface free energy changes upon increasing the layer thickness.

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